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TAMPERE UNIVERSITY OF TECHNOLOGY

MAIJA KUULIALA

RESEARCH AND DEVELOPMENT OF A BIODEGRADABLE SILICA-SILICA COMPOSITE FOR CONTROLLED DRUG DELIVERY

Master of Science Thesis

Examiner: Assisting professor Oommen P. Oommen
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Tutkimuksen toimeksiantaja oli DelSiTech Oy, jonka patentoitu silika-silika komposiitti soveltuu erilaisten lääkeaineiden, viruksien ja pienmolekyylien parenteraaliseen annosteluun. Työn tavoite oli tutkia eri komposiittiformulaatioita alhaisella mikropartikkelien massakonsentraatiolla (C_{mp} < 0.5 g/ml), joilla voitaisiin annostella tarvittaessa pienempiä lääkeannoksia. Tutkituissa komposiiteissa alennettiin silikahydrogeelin R-arvoa (veden suhde silikaattiin) hydrogeelimatriisin mekaanisten ominaisuuksien parantamiseksi. Onnistuneelle formulaatiolle määritettiin kolme kriteeriä: komposiitti on homogeeninen ilman selkeää faasierkautumista, injektoitavissa 27 G-neulan läpi ja lisäksi sen viskoelastiset ominaisuudet säilyvät ainakin kolme kuukautta. Ominaisuuksien arvioiminen ajan funktiona oli tärkeää, sillä alennetuilla R-arvoilla kondensaatioreaktion jatkuminen geeliytymisen jälkeen on mahdollista useiden kuukausien ajan, muuttaen komposiitin rakennetta ja injektoituutta.

Tutkimukseen kuului hydrogeelien valmistus alhaisilla R-arvoilla sekä komposiittien valmistus eri formulaatioilla ja kahdella eri menetelmällä. Komposiittien ominaisuuksia arvioitiin visuaalisesti ja lisäksi viskoelastisten ominaisuuksien säilymistä 14 päivän säilytyskokeella. Lisäksi kahden formulaation injektoitavuutta ja viskoelastisten ominaisuuksien kehitystä tutkittiin säilytyskokeissa injektiovoimakokeilla ja oskillaatiomittauksilla kolmen kuukauden ajan +37 celsiusasteessa.

Tutkimuksessa havaittiin alennetun R-arvon altistavan rakenteellisille muutoksille sekä hydrogeeleissä että komposiiteissa. Alennetulla R-arvolla ei saatu kompensoitua vähäistä mikropartikkelikonsetraatiota homogeenisen formulaation saavuttamiseksi: useimmissa formulaatioissa mikropartikkelit sedimentoituivat. Yksi homogeeninen formulaatio, R150 C_{mp}=0.3 g/ml onnistuttiin valmistamaan, mutta oskillaatiomittaukset ja visuaalinen tarkastelu osoittivat näytteiden muuttuvan geelimäisestä kiinteämmäksi ajan myötä. Lisäksi näytteiden injektoitavuus oli huono, kertoen faasiseparoitumisesta ja näytteiden heterogeenisyydestä. Tutkimuksessa ei onnistuttu valmistamaan homogeenista formulaatiota. Tulevaisuudessa voisi tutkia mikropartikkelien lisäämistä jo geeliytyneeseen hydrogeelimatriisiin tai hydrogeelin vahvistamista lisäaineilla matalien mikropartikkelikonsentraatioiden saavuttamiseksi.

ABSTRACT

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The mandator of this study was DelSiTech Ltd, whose silica-silica composite is suitable for parenteral delivery of pharmaceuticals and viral vectors. The goal of this study was to examine low microparticle mass concentrations in the silica-silica composite (Cmp < 0.5 g/ml), for delivering small doses. As the microparticle concentration was reduced, also the R-value of the hydrogel (water to silica alkoxide ratio) was lowered in order to improve the mechanical properties of the hydrogel matrix. A successful formulation was defined with three criteria: 1) the composite is homogeneous with no visible phase separation of hydrogel and microparticles occurring, 2) injectable through 27-G-needle and 3) it should maintain its viscoelastic properties for at least three months. Evaluation as a function of time was of great importance since continuous condensation reaction may go on in the composite for several months and thus alter the composite structure and injectability.

The study consisted of preparation of hydrogels with low R-values and preparation of composites with several formulations and two alternative preparation methods. Properties of the composites were evaluated visually and with 14 days storage test. Shelf-life studies included evaluation of viscoelastic properties with oscillatory measurements and injectability with injection force studies as a function of time for three months at +37°C.

Low R-value was observed to increase structural changes in the composite and hydrogel within time. Low R-value did not compensate the lack of microparticles to obtain a homogeneous formulation. Most of the studied formulations were heterogeneous and sedimentation of microparticles often occurred. One homogeneous formulation, R150 Cmp=0.3 g/ml could be prepared. However, oscillatory measurements and visual observation showed structural changes within time. In addition, injectability was poor which indicated phase separation and heterogeneity of the samples. Thus, a homogeneous formulation could not be prepared. Suggested methods to obtain low microparticle concentrations are addition of the microparticles to a gel or additives to the hydrogels matrix.

PREFACE

The past six months have been both a truly educational and rewarding phase in my life. Many people supported and assisted me during this project to whom I am most grateful. Firstly, I would like to sincerely thank all the staff in DelSiTech Ltd.: CEO, PhD Lasse Leino who accepted me to undertake this project, M.Sc. Panu Noppari whose teaching and guidance were of most significant value and all staff in DelSiTech Ltd. who gave both help and support during the whole project.

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Tampere, 5.2.2018

Maija Kuuliala

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ABBREVIATIONS AND NOTATION

23 G	Needle gauge (0.6 x 25 mm)
26 G	Needle gauge (0.45 x 25 mm)
27 G	Needle gauge (0.4 x 20 mm)
30 G	Needle gauge (0.3 x 13 mm)
C_{mp}	Microparticle mass concentration in silica-silica composite
G'	Shear storage modulus
G''	Shear loss modulus
tan(δ)	Ratio of shear loss modulus to shear storage modulus
In situ	In the original, or appropriate position
In vitro	Experiments conducted outside living organism
In vivo	Experiments conducted inside living organism
HAMC	Hydroxyapatite–methylcellulose
HCl	Hydrochloric acid
Hydrogel	Hydrophilic polymer containing dispersed water in solid network structure
LVE	Linear viscoelastic region
NaOH	Sodium hydroxide
PAA	Poly(acrylic acid)
PDLLA	Poly(D,L-lactide)
PLGA	Poly(lactic-co-glycolic acid
PEG	Polyethylene glycol
PEO	Poly(ethylene oxide)
PO407	Poloxamer 407
PVA	Poly(vinyl alcohol)
R-value	Molar ratio of water to silica alkoxide
Silica	Silicon dioxide (SiO2)
Sol	Colloidal suspension (solid particles in liquid)
TEOS	Tetraethoxysilane ($Si(OC_2H_5)_4$)

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1. INTRODUCTION

Controlled release systems have gained attention in recent decades due to their significant advantages compared to conventional drug delivery systems. In controlled release systems, drugs may be delivered to specific target site and with adjustable release profile. In addition, they may take effect for specific duration with reduced frequency of administration. [1][2] Drug bursts, which in here refer to rapid increase in concentration in bloodstream followed by quick reduction, are a great concern with conventional drug delivery systems, possibly resulting in systemic toxicity. A controlled release system aims to avoid bursts and moreover, a zero-order release profile with drug release in even doses can be achieved. [2]

A controlled release system consists of a protective cover that controls drug release and protects the drug from premature elimination [1]. Microparticles are a popular option in encapsulation and localized targeting of drugs [3, p.118–119]. However, combining microparticles in a protective matrix may provide more efficient and controlled drug release. Hydrogels possess excellent biomimetic properties and they have been extensively studied for drug delivery applications, also combined with microparticles. [4][5] The shear-thinning characteristics allows them to deform and flow under external load and retain their original shape upon relaxation [6][7].

A silica-silica composite represents a unique microparticle/hydrogel composite intended for controlled drug delivery. The silica-silica composite has been developed by the mandator of this study, DelSiTech Ltd., which is a Finnish company specialized in biodegradable silica-based controlled release systems. Both components of the composite, microparticles and hydrogel, consists of biodegradable amorphous silica. The main components of the hydrogel matrix are silica nanoparticles which are formed and aggregated in sol-gel process, constructing a typical three-dimensional gel network. Particles are formed in condensation of silica molecules which is highly dependent on pH [8, p. 103–105]. As nanoparticles form and aggregate, viscosity of the nanoparticle suspension (sol) increases. Mixing of microparticles and sol also drives the gelling process as the microparticles act as nucleating agents [9, p. 479]. As a conclusion, as gelation occurs, viscosity of the sol rises quickly enough to adequate level to prevent microparticle motion and sedimentation in the matrix.

Drug dosing is dependent on many factors including the size of a patient [10][11]. The ease of adjusting dosing is a great advantage of microparticles in controlled drug delivery systems: simply by reducing the amount of microparticles, a lower drug dose can be achieved. However, as they are embedded in a hydrogel matrix, reduction of micropar-

ticles will evidently alter the mechanical properties of the composite as well as the gelation time. Reduction of nucleation points increases gelation time, exposing microparticles for sedimentation. To prevent microparticle sedimentation, modification of the silica hydrogel matrix may provide a solution. Silica hydrogel R-value describes the molar ratio of water to silica alkoxide. By decreasing the ratio, the amount of water is reduced in the hydrogel structure, resulting in better mechanical properties and decreased gelling time. [8, p. 126][12] Reduction of microparticle mass concentration (C_{mp}) and hydrogel R-value at the same time may allow even distribution of microparticles in the matrix.

The goal of this study was to examine if a homogeneous formulation could be manufactured with R-value < 300 and C_{mp} < 0.5 g/ml, preferably < 0.3 g/ml. Homogeneous composite refers to a composite with no visibly distinct phase separation occurring. The goal was to use fresh, unaged sols in silica-silica composite preparation, indicating that microparticles and sol are mixed immediately after sol is prepared and pH is set to 6.2. The main objectives for a successful formulation were that they are homogenous, injectable through 27 G hypodermic needle and maintaining their viscoelastic properties for at least three months. The studied parameters included influence of low R-value on hydrogel structure and determination of lowest possible R-value still applicable in composite preparation, manufacturing composites with several formulations and conducting shelf-life studies on suitable formulations. Shelf-life studies evaluated viscoelastic properties and injectability of the composite as a function of time. Time is an important constraint since condensation may occur for months after gelation and result in structural changes [8, p. 358–362]. Viscoelastic properties and gel structure of the composites were evaluated by oscillatory measurements with small angle oscillatory shear. Injectability of the composites were studied with injection force studies. The injections were performed through 27 G needle.

2. THEORETICAL SECTION

2.1 Biomaterials in drug delivery

The concept of biomaterial is often used to describe materials that are in contact with biological systems. Even though biomaterials are used in many other applications than only medical ones, the focus usually lies on medicinal products that ones are also relevant in this study [13, p. 4][14][15, p. 1]. However, the definition of biomaterial and related concepts are somewhat miscellaneous. In 1987, the first consistent definition was established, defining biomaterials as "nonviable materials that are used in medical devices and are intended to interact with biological systems." [16] The definition is altering in different sources, and by removing the word medical from the definition, the definition of biomaterial is more diverse [15, p.1–2][16]. However, the main idea of biomedical biomaterials by 1999 definition is that the "material is intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function in the body."[16]

There are many ways to categorize biomaterials. They can be classified into four different groups based on their interaction in the human body: toxic materials which cause a harmful response and lead to tissue death, biologically inactive materials that are encapsulated by fibrous capsule in the body and are thus nearly inert, bioresorbable or biodegradable materials that dissolve while in contact with tissue and body fluids and bioactive materials that elicit a specific biological response that leads to the formation of a bond between tissue and the material. [17, p. 252][18]

Biocompatibility is an additional concept that needs to be defined. Unfortunately, there is no exact definition for biocompatibility other than in medical context, where it usually refers to materials having successful performance in their intended task. Biocompatibility is most often used to refer to tissue reaction to an implant or foreign material in which no great inflammation occurs. The concept of successful should be defined separately for each application. [15, p. 2]

The eventual disappearance of a material or device after being introduced into a biological system can be described as bioerosion, bioabsorption, biodegradation or bioresorption [19, p. 116]. Degradation refers to molecular breakdown or clearance of covalent bonds of a polymeric material and with addition of prefix "bio", it refers to degradation by at least partly biological activities [19, p. 116][20, p. 177][21]. Bioabsorption and bioresorption refer to total removal of polymer and degradation products by cellular activity, being also somewhat superfluous terms [19, p. 116]. Erosion refers to loss of

the material, monomers and oligomers leaving the polymer by complicated processes. However, polymer degradation is a key process of erosion. Thus, erosion can be understood as a process mediated by some other processes, for example degradation [21]. Erosion processes can be divided into two main categories which are surface and bulk erosion. Surface eroding materials lose material only from surface and keep their original shape. For bulk eroding materials, erosion and degradation are not restricted to surface [22].

Hydrogels represent a biomaterial group that have attracted attention in biomedical and pharmaceutical research. Especially, hydrogel-based drug delivery is attractive due to versatility of hydrogels and possibility of network tailoring [4]. In addition, a system with microparticles embedded in a hydrogel matrix offers a unique alternative for drug delivery [5].

2.1.1 Hydrogels

Hydrogels are hydrophilic polymers that have three-dimensional configuration and are capable of absorbing significant amounts of water or other biological fluids. They can swell in aqueous media [13, p. 19][23, p. 75][24][25]. The capability of absorbing water is attributed by the presence of hydrophilic groups, such as -OH, -CONH or $-SO_3H$ in polymer structures [25]. Water content in hydrogels is over 50 wt-% and can be even > 95 wt-% [26]. Degradation of hydrogels occurs by enzymatic activity, hydrolysis and/or dissolution [27].

The basic hydrogel microstructure can be described as a continuous polymer network. The continuous network is provided by interactions between the species [25]. Porosity and mesh size are important features of hydrogels which on the other side often play an important role in controlled release systems. Typically, the mesh size of biomedical hydrogels is around 5 to 100 nm at swollen state [13, p. 22][25][28, p. 230].

Hydrogels can be either physical or chemical hydrogels, based on how the crosslinks are formed [25]. Physical hydrogels form by aggregation, association, crystallization, complexation or hydrogen bonding or in other words by physical interactions. Physical gels are often reversible: they may dissolve by changing environmental conditions, such as pH, temperature or the ionic strength of solution [29]. Chemical hydrogels are constructed from chemical covalent crosslinked network in which covalent bonds join different macromolecular chains [23, p. 76][29].

In addition, hydrogels can be categorized based on their origin. They may be constructed of natural or synthetic polymers. Natural hydrogels may constitute from for example proteins, natural gums, cellulosic materials, hyaluronic acid, collagen and polysaccharides [30]. Synthetic hydrogels include for example poly(ethylene oxide) (PEO) and its copolymers, poly(acrylic acid) (PAA) and its derivates, poly(vinyl alcohol) (PVA) and

polypeptides [27]. Hydrogels made from natural polymers possess high biocompatibility but lack mechanical properties. On the other hand, synthetic polymers have usually more defined structures and their properties can be tailored [4].

To achieve customized functionality of hydrogels, several types of hydrogel composite materials have been developed. Nanocomposites or hybrid hydrogels are formed either from chemically or physically linked polymer network with nanoparticles or nanostructures. Nanocomposite hydrogels can be made from various particles, such as from polymeric nanoparticles, inorganic nanoparticles or carbon-based nanomaterials. As an advantage, they offer excellent formability of their properties and especially improved mechanical properties. [30][31] For example, the addition of silica nanoparticles to poly(ethlylene glycolide) (PEG) hydrogels was studied to enhanced mechanical properties and cell adhesion characteristics [31].

The use of hydrogels in drug delivery and tissue engineering is attractive since they possess some excellent biomimetic properties, including the ability to resemble physical properties of living tissues, low interfacial tension with water or biological fluids and excellent biocompatibility [4][24][25]. In addition, hydrogels have been proven to be suitable carriers for fragile biomolecules, such as proteins due to the lack of hydrophobic interactions which could possibly denature the proteins. Hydrogels can be also tailored at manufacturing state to response to external stimuli, for example to pH. It must also be noted that there are many design variables that affect to the drug release such as hydrogel composition, temperature, pH and ionic strength. [4] Some limitations exist in hydrogel drug delivery: homogeneity of drug loading may be difficult to obtain, especially with hydrophobic drugs. In addition, a high water content and large pore sizes may result in rapid drug release, even in some hours [5]. The diversity of applications may be simplified by using following categories: oral hydrogel systems, topical hydrogel systems, transdermal hydrogel systems, gastro-intestinal hydrogel delivery systems and ocular hydrogel delivery systems. In addition, there are other experimental routes for hydrogel-based drug delivery, such as vaginal and nasal delivery. [25]

2.1.2 Microparticles in hydrogel matrix

Microparticles can be efficiently used for localized targeting of drugs, referred to as particulate systems. Types of particles can be for example microspheres, nanoparticles or micelles. The microsphere size is from 1-1000 μm. For injectability, particle size under 100 μm is typically preferred. [3, p.118–119] Nanoparticles, on the other hand, are typically under 0,01 μm [32, p. 1]. Microparticles have generally large ratio of surface area and volume [20, p. 189]. It is possible to produce micro- and nanoparticles in different shapes and sizes by controlling the particle synthesis which increases their versatility [32, p. 16].

In drug delivery, microparticles are attractive for several reasons. For example, doses can be easily adjusted. In addition, administration is simple since the microparticles can be injected through fine needles which in turn increases the acceptance from the patients. Some challenges in use of microparticles include impossibility of removal after entering the body, large surface area that may promote aggregation and limited drug load level. There are multiple methods to encapsulate drugs into microparticles. For example, emulsion methods and spray-drying are typical for encapsulation. [8, p. 187, 192] Spray-drying will be the only one discussed here. It is a useful method for microencapsulation, since it offers the possibility to predetermine properties such as particle size and porosity, flowability and preservation of activity of heat sensitive pharmaceuticals [33].

Microparticles may be combined with physical hydrogels. The embedded substances do not only restrict to microparticles: liposomes, microgels, microemulsions, surfactant micelles and polymeric micelles can be entrapped in a hydrogel matrix [5]. Microparticle hydrogel composite can be considered as a multiphase system. Interfaces in multiphase systems can be described with structural, mobility and interaction characteristics. Structural characteristics include morphological and charge arrangements in material units that are taking part as micro- and nanoscale interlayers are formed. In principle, material morphological features in the interfacial region are affected by local shear forces, mobility of adjacent segments and interaction between involved groups of the phases. Molecular or segmental mobility can be restricted by a strong interaction. This in turn leads to a stable, amorphous structure at the interface. Local shear forces may also initiate orientation of the interface structure. Mobility is a second characteristic which is greatly affected by the interfacial structure and describes adhesion and molecular transport in the material. For example, adsorption is driven by amount of free energy. Materials with high surface energy, adsorption of water molecules is often occurring in normal conditions. Adsorbed layer may cause difficulties in processing, such as water release, decreased adhesion and aggregation. As a third category, interactions refer to physical or chemical bonds between phases and also activation and inhibition of chain growth or degradation reactions. [34]

The combination of hydrogel and microparticles has several advantages in pharmaceutical applications. Firstly, the degradable hydrogel controls the release of the drug. The hydrogels matrix acts also as an additional diffusion barrier and thus controls the drug release [5]. A drug may first diffuse to the hydrogel matrix and after that be released to its target site [26]. However, with small drug molecules, the hydrogel matrix may not affect to the release rate of the drug [36]. Additionally, high water content in the hydrogel may promote dissolution of certain particles [37]. By only using microparticles, there is a greater risk for burst release [20, p. 189]. Bursts and drug release will be discussed in chapter 2.4.2 Secondly, microparticles may be used to stabilize the composite [38, p. 283].

Microparticle composites have been utilized in pharmaceutical applications, constructed from different polymer combinations. Poly(lactic-co-glycolic acid) (PLGA) microparticles have been used in several studies, combined with collagen scaffold for parenteral administration, poloxamer 407 (PO407) gel, PVA hydrogel, chitosan-graft-poly(N-isopropylacrylamide (PNIPAM-g-chitosan) matrix and hyaluronic acid/methylcellulose (HAMC) hydrogel. [36][39][40][41] In addition, studies have been conducted with poly(D, L-lactide) (PDDLA) microparticles and PEG based copolymers [42]. The techniques of embedding microparticles in the matrix include lyophilization and casting, mixing in +4°C before matrix forms a gel and by adding microparticles to a solution with vigorous stirring (5000 rpm) [35][36][39][40]. In conclusion, microparticles are usually embedded in a solution that forms a gel.

2.2 Rheology and injectability

In principle, rheology studies the material properties and structure that cause the typical behavior of a material, for example its flow and deformation. More specifically, the effects on applied forces to the rate of deformation and flow are of great interest and the goals are to establish a relationship between applied forces and deformation and between rheological properties and material structure. One goal is also to establish models that help in qualitative or quantitative analysis of experimental results. [43, p. 2–4] However, rheological properties are not simple to predict since they are dependent both on time and measuring conditions [44, p. 18].

Rheology is an efficient method to characterize hydrogel degree of crosslinking, structural homogeneity or heterogenicity and molecular weight and it requires only small sample volume and good sensitivity. With hydrogels, rheological studies are usually conducted with small-amplitude oscillatory shear (SAOS). [45]

2.2.1 Shear stress and shear rate

To understand fundamentals of rheology and especially flow behavior, some parameters that affect the deformation and flow must be determined. The two-plates model is convenient in here since it is applicable in the studies (figure 1).

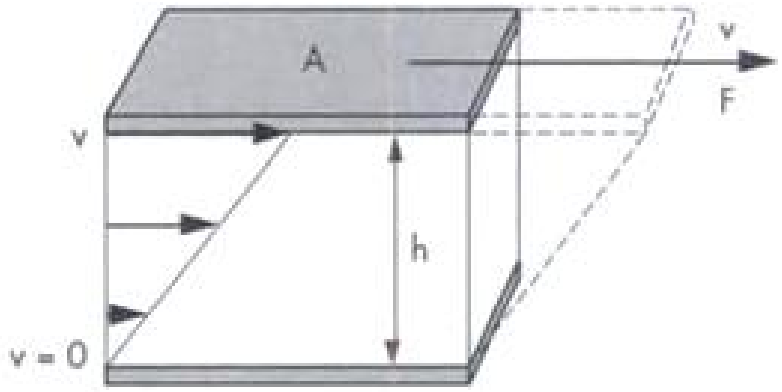


Figure 1. *Two-plate model. F presents the shear force, A the plate area, h the distance between plates and v the velocity. Modified from source [44, p. 19]*

The two-plates model consists of a moving upper-plate with a shear area *A* and the movement is caused by shear force *F*. The force moves the upper plate at velocity *v*, the lower plate is stationary. Distance between plates is *h* and the sample (liquid) is placed in the gap. [44, p. 19]

Shear stress (τ) can be defined as the ratio of shear force to shear area [44, p. 19]. The force is tangentially applied to the area. Shear stress causes the liquid to flow in a certain pattern, having the highest velocity near the upper plate and the lowest velocity near the lower plate. The velocity of the flow that can be maintained for a given force is controlled by the viscosity or internal resistance of the fluid. [46, p. 15, 21]

The model assumes that no slip occurs between the plates and the sample adheres to both plates. In addition, the flow between the plates is laminar, in the form of layer [44, p. 19]. Hence, the flow consists of thin liquid layers sliding on top of each other. A speed drop that occurs between the plates, by displacements of laminar layers is referred as shear rate (γ´) and it describes the ratio between the velocity and distance between the plates. [46, p. 16]

Thus, shear stress and shear rate describe the force to accelerate flow in a liquid and the velocity profile between two plates. Their ratio can also be abbreviated as shear viscosity. [44, p. 24] The connection between shear stress and shear strain and for viscosity and shear rate for different materials can be observed in figure 2. [46, p. 21]

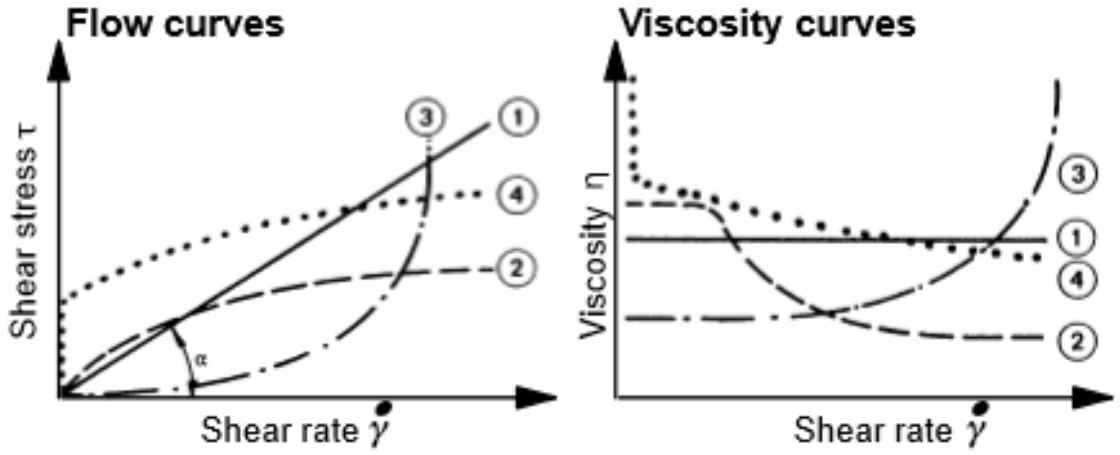


Figure 2. *Flow curve and viscosity curves. Curve number one represents a Newtonian liquid; the rest are non-Newtonian. Curve number two shows the behavior of a shear-thinning material. [46, p. 21]*

There are several models to describe liquid flow behavior. Generally, they are divided to Newtonian liquids that follow ideal flow behavior and Non-Newtonian liquids which do not. In general, for Newtonian fluids shear rate is linearly dependent on shear stress and viscosity is not dependent on shear rate, which is not the case for non-Newtonian fluids. [46, p. 21–22]

2.2.2 Viscoelastic and mechanical behavior of hydrogels

Elastic behavior of materials can be characterized as the capacity to store mechanical energy without any dissipation of energy. Another characteristic of elastic response is that materials exposed to a suddenly applied loading respond instantly and deformation remains constant. A viscous fluid in a non-hydrostatic stress-state has the ability of dissipating energy and not storing it. In addition, a viscous fluid flows steadily as uniform shear stress is applied. [47, p. 1] However, the behavior of a material can be a combination of the two models, simultaneously exhibiting viscous and elastic behavior and thus displaying a delayed response to applied load [44, p. 80][48, p. 300]. Viscoelastic behavior is especially prominent in polymers. An important aspect of viscoelasticity is that the mechanical behavior of a viscoelastic material is both time and temperature dependent [48, p. 300].

The ability to store and dissipate energy during deformation are described with Young's store modulus (E') and loss modulus (E''). Equivalently, the same characteristics in shear may be named as shear storage modulus (G') and shear loss modulus (G'') [48, p. 300, 306]. G' describes the elastic portion and G'' the viscous portion and in a gel, G' is dominating since the continuous phase is the solid phase [24]. Their ratio, G''/G', can be expressed as tan(δ) [43, p. 336] which in case of gel is <1 [26].

Mechanical properties of hydrogels are highly dependent on the network structure, especially on cross-linking density, degree of swelling and on environmental conditions [12][41]. Degree of swelling or extent of hydration is again, governed by the chemical structure of the polymer and degree of crosslinking [13, p. 20]. The mechanical behavior of hydrogels is commonly explained with theories of rubber elasticity and viscoelasticity. The response of hydrogels under mechanical stress vary significantly: response may be rapid, elastic recovery or a time-dependent recovery, indicating viscous behavior [49]. At swollen state, most hydrogels possess rubber-like behavior and may undergo fully reversible deformation under external load [12].

Mechanical strength can be altered by for example increasing crosslinking density by crosslinking agents or by changing the reaction conditions. Most of them aim to reduce the degree of swelling or the water content in the hydrogel. Thus, water content has a significant effect on the mechanical properties of the hydrogel. For example, if hydrogels are subject to water loss during testing, it can significantly influence the mechanical behavior. As temperature increases, water loss also increases which leads to subsequent changes in the hydrogel structure. This in turn leads to rising moduli values with temperature. [12]

However, hydrogel/ microparticle composites possess more complex behavior since they consist of several components [51, p. 587]. Viscoelastic properties of hydrogel/microparticle composite are mainly dependent on the rigidity of the matrix, the rigidity and volume fraction of the filler which are here the microparticles, and interaction between microparticles and matrix. [50][51, p. 585]. In case the rigidity of the particles is higher than the rigidity of hydrogel matrix, the particles have reinforcing effect on the composite. The stiffening effect of microparticles can be expressed as the ratio of G'-value of the composite to G'-value of the matrix. However, accurate modelling considers an idealized composite material: microparticles should be of approximately the same size and uniformly distributed. This means that the composite material is macroscopically homogeneous. [50]

Viscoelastic behavior can be explained for many materials by generalized models, for example Maxwell model for viscoelastic fluids and Kelvin model for solids [47, p. 19]. In addition, the complex behavior of composite gels can be modelled with several models, including Van der Poel-Smith's model and Kerner-Lewis model [42]. Flow behavior can be simplified for interpretation with mathematical models for curve fitting, such as the Power-Law for shear-thinning fluid. [44, p. 53].

2.2.3 Shear-thinning hydrogels and their injectability

As a subcategory of non-Newtonian fluids, some fluids are pseudoplastic (shear-thinning) and their viscosity drops at higher shear rates which for example allows injectability of a material [46, p. 21]. The shear-thinning flow behavior is not uniform at

very low to very high shear rates. At low shear rates, the Brownian motion of molecules causes the particles to be in random positions regardless of shear orientation. Thus, at very low shear rates, shear-thinning liquids behave similarly to Newtonian liquids and have a zero shear viscosity ($\eta 0$) which is independent of shear rate. When the shear rate increases, at some point the shear induced molecular or particle orientation begins to dominate the Brownian motion which causes the viscosity to drop significantly. At extremely high shear rates, the viscosity will begin to approach a constant level. At that point, a perfect orientation is reached and the behavior is no longer shear-thinning. [46, p. 23]

Injectability may be defined as the ratio of mass of injectable material able to be extruded from a syringe until a maximum force is reached to the initial mass of the material in the syringe [52]. Shear-thinning hydrogels can be injected directly through a syringe and then undergo a rapid sol-gel transition at their target site. Shear-thinning hydrogels in biomedical applications may constitute from peptides, proteins, hydrogel blend or colloidal systems, consisting on colloidal particles [6][7]. Hydrogels may form crosslinks *in-situ* which refers to gelation at their target site. Thus, the injectable product is a solution, not a gel. Formation of crosslinks may be induced by temperature, pH, ion-concentration or hydrophobic interactions. In addition, hydrogels that gel *in vitro* may be used as injectable material. Some physical hydrogels may exhibit viscous flow under shear stress and then time-dependent recovery once shear stress is removed. [6] Shear thinning hydrogels may orientate during injection in a way that they possess shear-thinning behavior as external force is applied to the plunger of the syringe and flow more easily [53]. Shear-thinning behavior arises from the microstructure of the material and the effect of shear in different materials is shown in figure 3 [54].

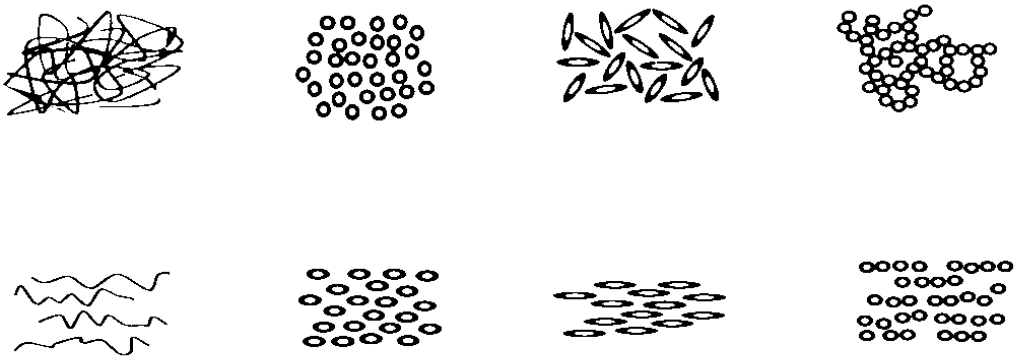


Figure 3. *Microstructures at rest shown above and under shear shown below. From left: polymer chains disentangling, emulsion droplets reorganizing and deforming, elongated particles aligning with the flow and aggregated structures breaking down to primary particles. [54]*

The case on far right in figure 3 presents the situation in physical hydrogels. The nanoaggregates go through reorientation and break down to primary particles in injec-

tion [7][54]. Viscosity drops significantly since particle interaction decreases and free space between dispersed components increase because of reorientation [55]. Once such materials are introduced to physiological conditions, they reorganize and retain their hydrogel structure [53]. Viscosity is quickly retained to the original level as shearing is slowed down or terminated [46, p. 23]. Recovery time is important in drug delivery applications. If the recovery time is too long, sedimentation or leakage of the encapsulated substances may occur [6].

In successful injection, three requirements must be fulfilled: no blocking of the needle, even flow of the injected product and suitable force. Needle gauge has a significant effect on injectability since the shape, inner diameter, surface finish, length and shape of the opening are all influential to the flow of the product. In addition, variables such as viscosity affects to injectability. [55] The applied force in injections dissipates in three ways including overcoming the resistance force of the syringe plunger, imparting kinetic energy to the material and forcing the material through the needle. Additionally, force is also required for administration to any medium, in here to the subcutaneous tissue. [56] Force is an important variable in injections. Generally, 80 to 100 N can be considered as a maximum finger pressing force of an adult, depending also on gender and age [57]. Viscosity of the material is significantly affecting to the injection force. In protein solutions, high concentrations have been studied to increase the injection force [56][58].

As mentioned, some composites exhibit complex mechanical but also rheological behavior. A two-phase system is especially fragile to phase separation during injection. In pharmaceutical applications, phase separation is a common phenomenon in extrusion of biphasic pastes. Some mechanisms of phase separation have been recognized during injections in which the pressure is the driving factor. The two main categories are filtration in the needle and filtration in the barrel. Phase separation may be induced by filtration in the needle, where the pressure causes the liquid to flow along the needle more rapidly than the solid phase. This causes regions of high solid volume fraction. Filtration may also occur in the barrel: the pressure exerted by the plunger on the material causes the liquid phase to quickly flow and redistribute. In contrast, the solid phase integrates and may be present as static regions, especially on both sides of the barrel exit. [52] In microparticle composites, as microparticle concentration is lowered, the mechanical properties of the composite weaken which decreased ability to withstand applied stresses also in injection [50].

2.2.4 Oscillatory measurements

Oscillatory measurements allow to determine elastic and viscous material properties with non-destructive methods. The principle is to induce forced oscillating stress by rotation with a small angle to the left and to the right and to study the influence of the frequency on storage and loss modules [44, p. 114][59, p. 122]. A viscoelastic material

responses to oscillating strain with stress that lags by phase angle (δ). This is presented is figure 4.

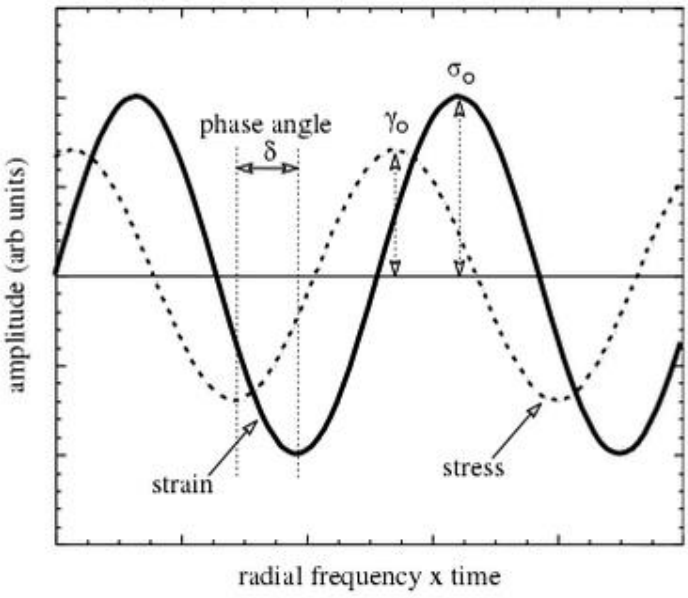


Figure 4. *Viscoelastic material response to oscillating strain. The stress is out of phase with the strain by phase angle (δ). [60, p. 100]*

The stress is out of phase with the strain. Hence, a portion of the energy is saved and in phase and dissipated energy will be out of phase with the applied strain. A viscoelastic material elicits a phase shift between 0-90°, from 90° at low frequencies to 0° at highest frequencies. Storage modulus and elastic modulus can be applied to describe the oscillation system with the following equations:

$$G'(\omega) = G^*(\omega)\cos(\delta) \quad (4)$$

$$G''(\omega) = G^*(\omega)\sin(\delta), \quad (5)$$

where ω is the radial frequency and G^* is the complex modulus or ratio between stress and strain. [60, p. 100–104] The applied stress causes the material to undergo deformation (γ) [59, p. 80].

Oscillatory measurements are usually conducted in the linear viscoelastic region (LVE). At the LVE the G' and G'' are independent of stress or strain values [59, p. 82]. In LVE, the sample retains its structure. If the stress is high enough, the sample is deformed and the internal temporary bonds of aggregates are destroyed. In addition, shear-thinning begins and a major part of the energy is irreversibly lost as heat [46, p. 133]. The LVE limit can be determined by amplitude sweep (figure 5) [61].

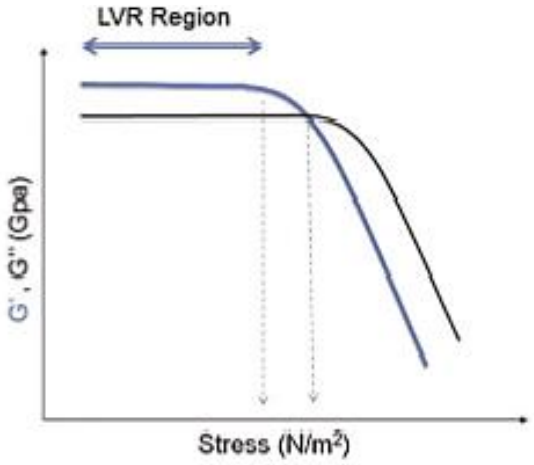


***Figure 5**. Illustration of the LVE (abbreviated as LVR) region. The LVE region ends when the plateau of G' values end. [38]*

As seen in figure 5, at the LVE (abbreviated as LVR) G' values are in a plateau and then decrease as stress is increased. The sweep can be done with a controlled deformation (CD) program where the instrument will detect the necessary stress value to accomplish the requested deformation and thus plot G' and G'' values as a function of deformation. [59, p. 80]

There are several oscillation measurements modes that can be conducted to characterize materials [59, p.77–90]. Rheological properties study of hydrogels can provide information on their structure and on the viscoelastic properties as well as the network parameters within the hydrogels. However, it must be noted that rheological behavior of hydrogels is also strongly dependent of environmental conditions [62]. Oscillation frequency sweep is a typical procedure to characterize structural conditions of gels [51][59, p. 80]. In frequency sweep, the G' and G'' values are plotted as a function of frequency at constant temperature [61].

In gels, the three-dimensional network forms a continuous structure and in frequency sweep and they should be showing G'>G'' at the whole frequency area. Elastic behavior dominates the viscous one. In addition, the G' and G'' curves are often almost parallel curves, showing only a slight slope. [44, p. 141]

In addition, homogeneity of the samples is an important measuring constraint in rheology. Homogeneity of the sample meets the requirements of rheological studies when the sample shears uniformly. This can be problematic in case of dispersion or suspensions but also for composites [46, p. 31–32]. Suspensions and filled polymers, for example, cannot be considered as homogenous in principle [43, p. 40]. Phase separation might occur at high shear rates and cause a liquid layer on top of the sample [44, p. 32]. Inhomogeneous material may lead to irregular distribution of stress and strain throughout the material [50].

2.3 Silica-silica composite

Silica-based drug delivery systems have been rapidly developed during the past decades, including applications such as targeted drug mechanism studies, drug kinetics marker in pharmacological research and evaluation of the effectiveness of the drug release in diagnosis and therapy [63]. In addition, an advance of silica-based controlled drug delivery systems is that they can be functionalized to response environmental changes such as to changes in temperature, pH, magnetism and luminescence [65].

Silica microparticles have also been combined with organic materials for pharmaceutical applications. However, combining the microparticles with a matrix that is the same material but owing a different state is a clear advantage in terms of toxicology, product development and function. If the same chemical substances are for preparing the hydrogel and the microparticles, the impurities stay low and there is no need to study the combination effects of different substances. Biodegradation mechanism is simpler to predict than when using a combination of silica and organic polymers. [26]

2.3.1 Silica chemistry

Silicon is the second most ample element on Earth and is mostly found as materials containing silicon, referred to as silicates. The ubiquitous presence of silicon occasionally causes confusion in the terminology. Silicon refers to the element and silicone or polysiloxanes to a family of polymers with silicon-oxygen back-bone. Silicon compounds are synthetic polymers where carbon atoms are connected to silicon atoms. Silica refers to silicon dioxide (SiO_2) and to chemically combined forms. [13, p. 109-110]

Structurally, silica consists of a three-dimensional network in which every corner oxygen atom in each tetrahedron is shared by an adjacent tetrahedral [66, p. 426]. Silica contains Si-O bonds, that are relatively stable compared to other Si-X bonds. Silica may be present in both crystalline and amorphous forms, crystalline silica forming regular structure from SiO_4 units and amorphous silica by random packing of SiO_4 units [67, p. 10]. Hydroxylation of the surface of amorphous silica is an important phenomenon. A fully hydroxylated silica particle is presented in figure 6.

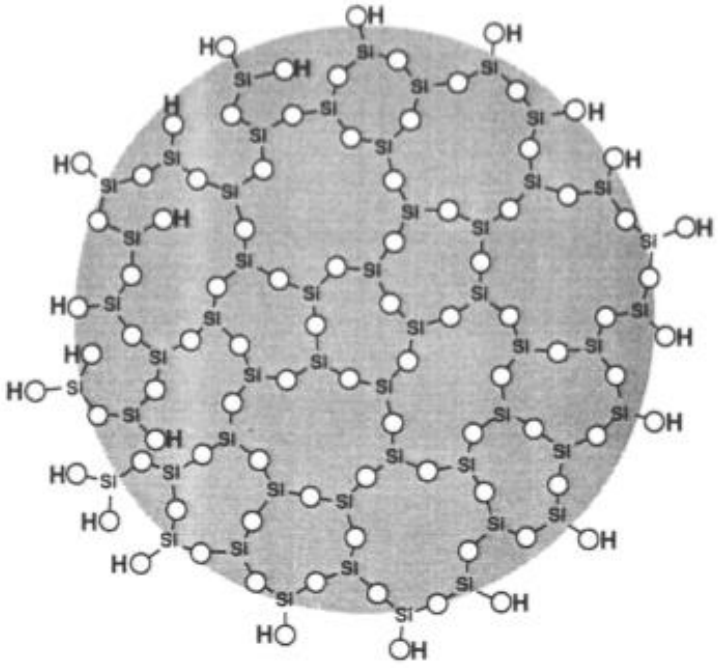


Figure 6. *A model of a fully hydroxylated silica particle. The figure is two-dimensional and the fourth oxygen coordinated with the Si-atom is behind the particle or towards the reader. [67, p. 17]*

A sufficient amount of OH -groups causes the hydrophilicity of the silica surface. The surface OH-groups mainly cause the adsorption of water molecules [67, p. 22]. Moreover, the $R_3Si–O$ bonds are called silanol groups [68]. Silanol groups can be further categorized but will not be discussed here.

Polymerization of silica in aqueous systems differs from other organic polymers formed by simple condensation. Instead of linear polymer formation, the monomers form particles which slowly grow and finally aggregate and link into chains [8, p. 99–100][53]. Silica hydrogel belongs to physical hydrogels since silica nanoparticles aggregate and crosslinks are provided by van der Waals interactions [53]. Polymerization will be discussed in chapter 2.3.2.

Nanoscale particles that form the silica hydrogel network by aggregation are colloidal [53]. Colloidal silica refers to dispersed systems in which silica is the disperse phase. Colloidal particles are defined as particles with a size sufficiently small ($\leq 1\mu m$) not to be affected by gravitational forces but sufficiently large that short-range forces, such as van der Waals attraction and surface forces are dominating the interactions. The particles exhibit Brownian motion which means the random movement of particles, driven by the momentum of random collisions with molecules of suspending medium. Hence, colloidal dispersion is defined as a system in which particles of colloidal size of any phase are dispersed in a continuous phase owning a different composition. [8, p. 2][67, p. 1] In case of silica microparticles, the system is not colloidal anymore. The nanoag-

gregates form a microparticle which is large enough to be affected by gravitational forces [53].

2.3.2 Sol-gel processing

Sol is defined as the colloidal suspension of solid particles in liquid [8, p. 2]. Colloidal suspensions are biphasic systems where the interparticle forces of discrete phase are in significant role in governing system properties [50]. Hence, in a silica-silica composite, the silica nanoparticles represent the solid particles. In a sol, the liquid phase is the continuous phase [53]. Gel is defined as a colloidal dispersion in which solid phase is the continuous phase and liquid is the dispersed phase [26]. In gelling, the silica nanoparticle aggregates to form a continuous network [53].

Sol-gel derived silica is prepared from metal alkoxides which have an organic ligand attached to metal atom. Metal alkoxides are often used as precursor since they react easily with water. The most common tetraalkoxysilanes that are used as precursor are tetraethoxysilane ($SiC_8H_{20}O_4$) and tetramethoxysilane ($SiC_4H_{12}O_4$), often referred as TEOS and TMOS, respectively. [8, p. 2–3, 112]

The sol-gel processes of silica can be divided in three phases and two main reactions: hydrolysis followed by condensation in two steps. The system can be identified as a two-step inorganic polycondensation. Three equations are presented below. [64]

Hydrolysis:

$$Si–OR + H_20 \rightarrow Si–OH + ROH \quad (1)$$

Condensation:

$$–Si–OR + Si–OH \rightarrow Si–O–Si + ROH \quad (2)$$

$$–Si–OH + Si–OH \rightarrow Si–O–Si + H_2O \quad (3)$$

At the first step, hydrolysis occurs, performed with a presence of a catalyst to obtain the most rapid and complete process. Generally, mineral acid or ammonia are used. The alkoxide groups (OR) are replaced with hydroxyl groups (OH). Condensation reactions involve silanol groups producing siloxane bonds (O–Si–O) and as a byproduct alcohol (ROH) or water. Alcohol is used typically as homogenizing agent since both water and alkoxide are immiscible. However, the alcohol byproduct is enough to homogenize the two-phase system. Condensation aims to maximize the amount of Si–O–Si bonds and thus to minimize the number of terminal hydroxyl groups. Hence, monomers are added to rings which form the three-dimensional structure. [8, p. 102, 109, 116]

In reactions (2) and (3), the silica particles are formed by polymerization of the monomer. For hydrogel formation, two additional steps are required, which are the growth of the particles and then attaching of particles to form chains [8, p. 100]. As mentioned, silica only forms oligomers and does not polymerize linearly. It takes approximately 20-50 silica molecules to form a particle which is approximately 2-5 nm in diameter. The particles are constantly formed in liquid and moved by thermal radiation. The particles hit each other and aggregate [53]. In addition, small particles below some critical value redissolve due to high solubility and allow larger particles above that radius to grow even more. This causes size distribution of particles in the solution and the phenomenon is called Ostawald ripening [69]. The silica backbone is formed as aggregated particles form clusters [64]. The aggregation continues and formation of branched structures or clusters goes on until a gel is formed [71]. Gel-point refers to the point where elastic properties start to dominate: this can be observed in rheological measurements [26]. The process is presented in figure 7.

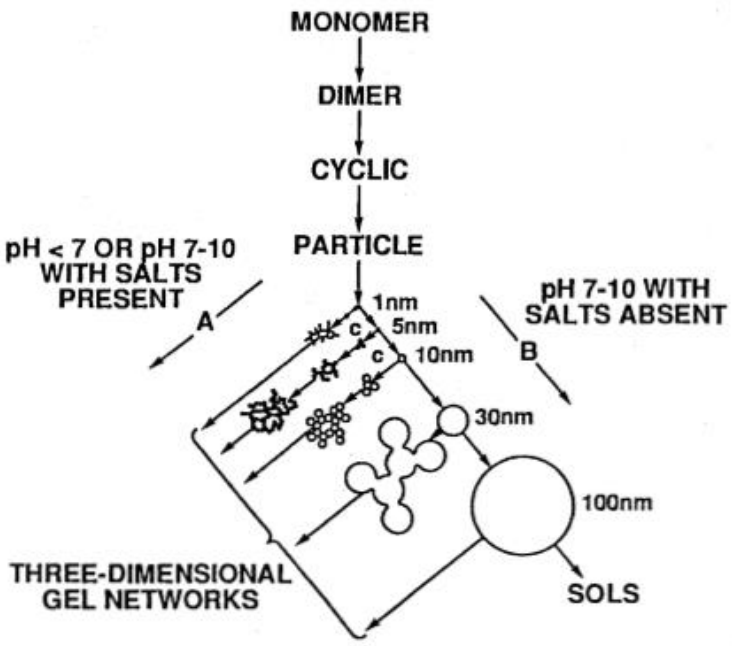


***Figure** 7. Sol-gel process of silica. The process B particles only grow with decrease in number. Considering this study, the process goes as process A and particles aggregate. [8, p. 102]*

The resulting structure can be described as fractal: the structure is formed by random condensation and aggregation. Aggregation typically leads to the formation of clusters with highly branched fractal flocs [50]. The microstructure of silica hydrogel can be further characterized with three characteristics which are the fractal dimension (*D*), the average cluster size (*Rg*) and the mean diameter of primary particles constituting the

structure (*a*). The fractal dimension thus describes the mass distribution in the volume. [70]

The microstructure parameters may be altered by changing silica concentration or pH-value of the polymerization process. Polymerization can be divided into pH domains (figure 8).

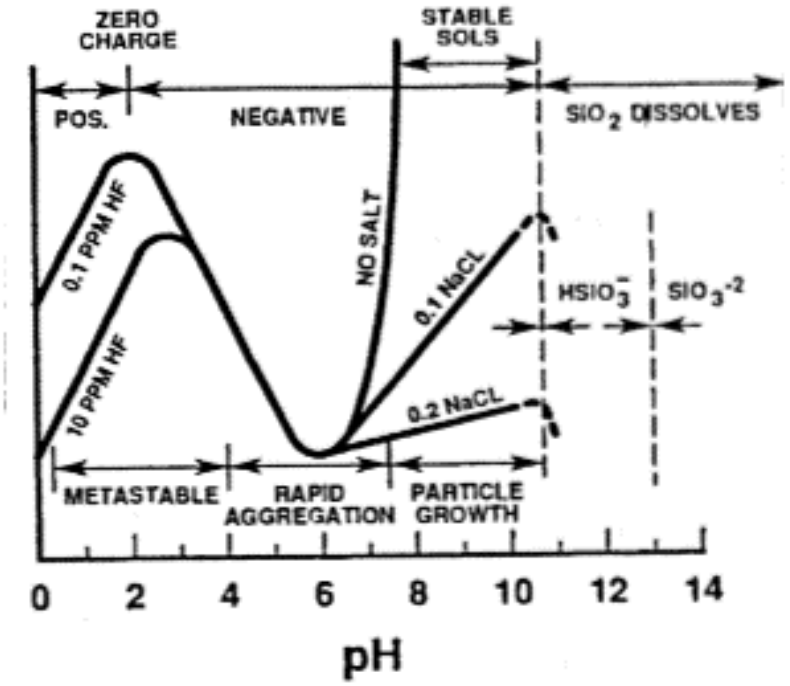


Figure 8. *pH dependence of silica polymerization. In pH=2, silica is metastable and in approximately pH=6, the maximum aggregation speed is reached. [8, p. 104]*

In pH 2, silica appears to be metastable and the reaction proceeds very slowly. This is because both the point of zero charge and the isoelectric point where electric mobility of silica particles is zero are both in pH level 1-3 [8, p. 103–104]. At the isoelectric point, the interaction of silica species is very weakly attractive [72]. After pH 2, aggregation starts to proceed rapidly and reaches the maximum speed at approximately pH 6. After pH 7, growth occurs as monomer addition to more condensed particles, instead of aggregation and condensed particles are likely to be ionized. [8, p. 103–105]

The effect of increasing pH for the microstructure is shown in figure 9.

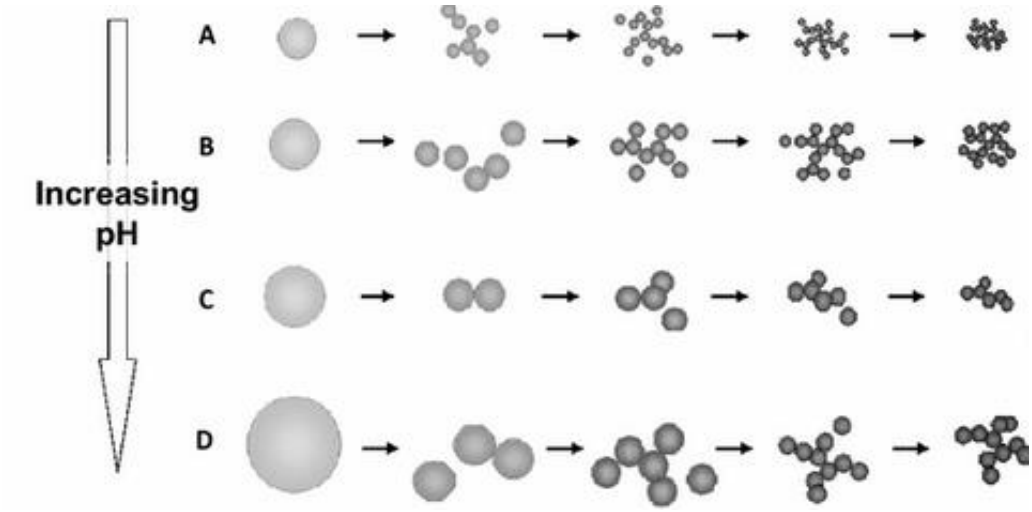


Figure 9. *Effect on pH to the cluster formation. Situation A and B represent low pH values in which parameter a keeps approximately constant. Parameter a then increases with pH (situations C, D). Situation C represents the pH value of approximately 6 where condensation proceeds rapidly. [70]*

As pH increases, the radius of elementary particles increases. Thus, smaller and denser structure having low *D* and *Rg* are obtained at low pH. Low electrostatic repulsion allows small particles to form a dense structure. As particle size grows, surface charges increase and cause more open structure with increasing *Rg*. If pH rises even more, strong electrostatic repulsion causes again more open structure. [70]

The rate of condensation reaction significantly increases as pH increases. However, electrostatic repulsion only slightly increases with pH. Thus, at pH 6, the frequency of particle collisions significantly rises. This increases the number of incipient clusters and decreases the average number of particles forming the primary structure. This results in low *D* and *Rg* at pH values near pH 6. [70] In addition, at higher concentrations, particle crowding produces increased probability of collision between particles and thus the polymerization proceeds faster [50].

Silica concentration has a clear effect on resulting hydrogel structure. The R-value indicates the amount of water in the hydrogel structure. Low R-values yield weakly branched, "polymeric" sols. High R-values indicate higher amount of water and yield to highly condensed, particulate sols. High R-values promote hydrolysis and the reaction may go into completion. A fully hydrolyzed monomer is tetrafunctional and has thus two reactive hydroxyl groups which leads to branched structure upon polymerization. With low R-values, hydrolysis is not complete and fewer reactive groups are present in the structure and thus only little branching is present. However, as R-value is increased and while maintaining a constant solvent to silicate ratio, the concentration of silicate reduces which increases the time for sol to form a gel. As R-value is lowered, more reactive species (silica molecules) are present in the sol and the frequency of particle collisions rises. [62, p. 5–6, 112, 126][70]

In polymer composites, the interplay between filler and matrix often results in nucleation [73]. In sol-gel processing, micro- and nanoparticles have been studied to provide a heterogeneous seed of nucleation and speed up the structure formation [9, p. 479]. Here, in silica-silica composites, the microparticles act as nucleation seeds and gelation proceeds more rapidly as microparticle concentration (C_{mp}) is increased.

2.3.3 Aging of the composite

Although silica composites seem stable after gel formation, chemical activity continues inside the composite. Aging refers here to the processes of structural change and change of properties after gelation or in other words after the gel-point has been reached [8, p. 9]. The driving force for structure transformation can be derived from entropy of the structures [67, p. 603]. The structure of silica nanoaggregates is rigid. Hence, in order to decrease entropy, gradual dissolution at the highest places of entropy and depositing dissolved monomers to places with lower entropy increases the solubility and structure reorganization [67, p. 603]. Processes that occur after gelation can be divided into three categories: polymerization, coarsening and phase transformation [8, p. 358].

In polymerization of silica, condensation continues after gelation due to labile hydroxyl groups which will eventually stiffen the network. Condensation may continue for months after gelation and the rate of reaction is dependent of pH, temperature and condensation. It has been observed that G'-value, which increases as new bonds by condensation of hydroxyl-groups are formed, rises for longer time as R-value is decreased. This is due to higher R-values being more completely hydrolyzed and thus the possible stiffening occurs faster. Another phenomenon that may be caused by condensation is syneresis or shrinkage of the gel network, resulting in release of water from the pores. Syneresis may cause cluster formation which in turn causes liquid areas in the gel. Aging may also result in further hydrolysis or in reverse reaction which is re-esterification. Re-esterfication can be prevented by introducing excess water in the structure. Coarsening is a dissolution process driven by differences in solubility. It causes smaller particles to dissolve and reprecipitate. The average pore size increases and necks are growing larger between particles (figure 10). [8, p. 358–362, 389]

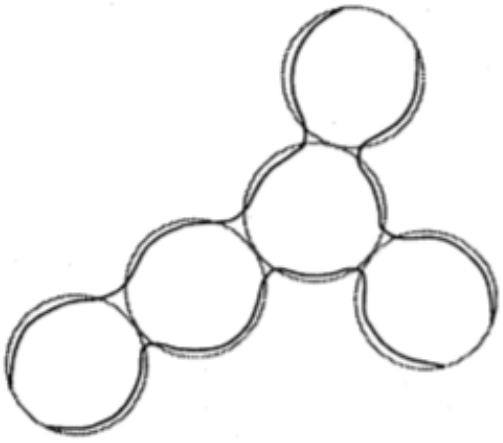


Figure 10. *Dissolution of particles. The dissolution will result in larger necks between particles (presented as the black line) and thus stiffen the network. [62, p. 36]*

The final category of aging is phase transformations. Segregation of liquid in two or more phases may occur. [8, p. 364]

Thus, aging has significant effects on the composite properties. Aging of the composite can be accelerated by high pH values or temperatures and the presence of fluorine anions [67, p. 603]. Fluorine anions catalyze the dissolution of silica and enhance the rate of aging and higher temperatures have been studied to rapidly increase the porosity of silica. High pH values accelerate solubility and rate of dissolution of silica. In addition, water content has an important role in aging. Organic liquids can retard aging by inhibiting condensation reactions and adsorbing silanol groups. [8, p. 364–368]

2.4 Silica in controlled drug delivery

The main objective of both drug delivery and drug formulation is to provide efficient drug administration at a therapeutic concentration to a specific site of action [74, p. 58]. Drug substances are rarely administered at their natural state: they require protection and carriers to achieve the benefits for a patient [75, p. 9]. Thus, for achieving better therapeutic effect of a drug, it is not always necessary to increase the drug concentration but to rather develop an optimal drug delivery system [76, p. 24]. Controlled release systems aim to protect the drug from premature elimination, assist the drug to pass physiological barriers and to target the drug the desired area with reduced frequency of administration [1].

Compared to conventional drug delivery systems, for example tablets and capsules, controlled release systems differ in the rate of release. Conventional drug delivery systems cause bursts of drug. These variations may cause toxic plasma drug levels or result in poor effectiveness. Most controlled release systems aim to maintain a constant drug

level in the bloodstream after the rapid concentration increase in the beginning that is on the effective range. [2]

2.4.1 Silica in biological systems

Silica has been studied in several applications in biomedical field, for example bone-repairing devices and drug delivery systems [77]. During the last few decades, silica has been used for both soft and hard tissue regeneration [84.] Applications of amorphous silica do not restrict to biomedical applications: synthetic amorphous silica has been utilized in the manufacture of products such as paints, cosmetics, and food as additives and fillers [78].

Biocompatibility of silica is greatly dependent on the microstructure. Inhalation of crystalline silica has been studied to cause an inflammatory reaction and additionally, chronic exposure leads to lung fibrosis and can progress into silicosis [78]. In addition, crystalline silica has been studied to induce lung cancer [79]. Toxicity of amorphous silica has raised some controversial evaluations although generally it is considered non-toxic [64][79].

In case of silica nanoparticles, toxic effect on cells rests upon cell type and cell line [79]. Macrophages have been studied to display sensitivity to amorphous silica nanoparticles, which relates to ability for phagocytosis [78]. However, nanoparticle size also influences the cytotoxicity since the size affects to cellular uptake [78][79]. Biocompatibility of biodegradable silica with white blood cells has been studied by Puskala. The silica sol and the silica hydrogel has been studied not to activate the white blood cells but to inhibit their activation. Thus, combining silica microparticles and silica hydrogel, an improved biocompatibility compared to plain microparticles can be achieved. [80]

2.4.2 Principles of controlled drug release

Practically, most controlled release systems aim at achieving the zero-order release profile with constant drug concentration in the bloodstream. However, a rapid increase of plasma drug level before it reaches a stable profile is present in most of the controlled release systems. This can be referred as a burst in drug release. In injectable hydrogel systems, drug bursts can be caused by unsuccessful encapsulation of the drug since polymer precursors do not set immediately. Other possible reasons in hydrogel systems are processing conditions, sample geometry, host and drug interaction and surface characteristics of the host material. [2] Preparation processes with sudden phase transformations such as spray-drying may cause relatively large drug amounts to concentrate near the matrix surface [26]. Drug bursts may have negative effects such as local or systemic toxicity [2]. However, drug bursts might be beneficial in some cases. Some controlled delivery systems aim at variable release in which doses are intentionally not identical, for example in the treatment of hypertension. Still, the systems are more diffi-

cult to develop [81, p. 34–35]. Bursts can be controlled to some extent such as by chemical surface or pore size modifications [26].

There are several routes of administration for drugs and also several dosage forms available [75, p. 14–15]. Parenterally administered drugs are injected through a needle to various sites and to various depths. Parenteral administration aims to avoid the physical and enzymatic degradation of drugs before they reach their target site [74, p. 322]. Three main routes in parenteral administration are subcutaneous injection beneath the skin, intramuscular injections into muscle and intravenous injections into veins. Another more specific example is an intraocular injection into the eye. Drugs in parenteral administration are usually either in a form of a solution or a suspension. Suspensions need to dissolve before the drug can be absorbed and therefore produce a response slower than solutions. [75, p. 20]

For parenteral administration, injectability or ministration via a surgical administration apparatus, for example a needle, a catheter or a combination of these is necessary [26]. Subcutaneous injection is usually performed through a 23–26 G needle, even finer needle is mostly applicable [55][82] and intraocular injection through a 27–31 G needle [83]. The finer the needle, the more comfortable it is to a patient and less tissue damage is caused. For sensitive tissues, such as the eye, a fine needle is necessary. However, a fine needle sets a challenge to the injectability of the product. The finer a needle is, the more force is needed to push out the injectable product. [62] Thus, there must be a balance between injectability, tissue damage and pain level of the injection.

There are several mechanisms how drug release to the body is controlled, as mentioned before. However, with silica-silica microparticles and encapsulated drugs, erosion meditated by dissolution is the main mechanism and will be the only one discussed here [33][51]. Dissolution controlled release systems have drugs coated or encapsulated inside of slowly dissolving matrices, referred as monolithic system or membranes and reservoir system, respectively [1]. Dissolution involves transfer of the solid drug to the surrounding medium. The medium may be tissue, water or polymer. Solubility of the drug in a medium is defined as the concentration of the drug in the medium at saturation point. Solubility decreases as melting point of the drug increases and is thus a thermodynamic property. Hence, dissolution rate is dependent on solubility and particle size and is a kinetic property. [84, p. 30] With monolithic systems, the drug aggregates are distributed throughout the polymer matrices and dissolve when the matrix dissolves. In reservoir systems, the membranes have different dissolution rates and release the drug at different times. It is important to notice that controlled release mechanisms do not work only separately. The system is practically never dependent on only one mechanism but they overlap or are a combination of several. They may also dominate other mechanism. [1]

2.4.3 Silica degradation and drug release

Biocompatibility and degradability of sol-gel derived silica matrices as well as their tailorable properties make them attractive as drug delivery systems [76]. In addition, composite hydrogel delivery systems have attracted notable attention in advanced drug delivery, although some challenges such as accurate mathematical modelling are yet to be solved [85].

The release of drugs from amorphous silica microparticles is mainly governed by silica degradation and bulk erosion meditated by dissolution [18][64][86]. Diffusion of drug molecules has not been shown to be significant since the silica microparticles are dense enough to prohibit it [18][33]. Degradation of the matrix is governed by hydrolysis of siloxane bonds through the gel network [88]. It has been shown that *in vivo* silica dissolves by hydrolysis into body fluids as silicic acid without any additional steps. The silicic acid is removed mainly through urine [33]87][88]. However, biodegradable systems are not usually following purely ideal erosion models but are a combination of bulk and surface erosion models [33][88]. In addition, the structural variability of amorphous silica also makes it difficult to establish the accurate mechanistic models of dissolution [89]. It should also be noted that degradation or molecular breakdown of the matrix is governed by the chemical composition and morphology as well as environmental conditions and device properties which should be considered [20, p. 177].

Usually, surface eroding thin polymers are following zero-order kinetics. It is possible to achieve zero-order kinetics with bulk-erosion with suitable matrix properties such as water and drug diffusion and polymer swelling [88]. Silica microparticles have been found to follow zero-order release [72]. This is because the solubility of silica in aqueous solution is low. Silica does not dissolve in silica saturated conditions. As silica microparticle pores are introduced to aqueous solution, local saturation occurs fast. This limits dissolution rate inside the pores slow. [26][33][86] Some variation can be observed in the release results which is shown most obviously in the initial burst. The composite structure has been observed in rheological studies to integrate as saturated silicic acid in the pores and presence of nanoparticles enhance the condensation on the microparticle surface. The effect of the integrated structure is likely to be strongest at the beginning of dissolution. Integrated structures make diffusion paths longer which in turn affects to the degradation of silica. [26]

Microencapsulation techniques are not suitable for all polymers and drugs. The advantage of spray-drying is that drug loss is not a major issue since it involves gas in particle dispersion, not solvent. However, controlling the capsulation is difficult. Furthermore, uncoated drug might occur. [20, p. 188] The drug burst effect occurs if encapsulation is unsuccessful [33].

There are two ways to control the release of drugs from silica particles, either by controlling the dissolution rate or the degradation of silica. The dissolution of silica has been studied to be relatively easy to control by adjusting some conventional sol-gel parameters: R-value, amount of solvent, catalyst concentration and the process parameters, for example aging and drying [87]. An increase in the R-value yields to increasing specific surface area, which also partly controls the release of drugs. Decreasing R-value has been observed to decrease the release rate with silica xerogels, which have more condensed structures [88].

2.4.4 Composite formulations for low microparticle concentrations

Drug formulation and medical device development have many variables affecting to the functionality of the product. Response to drugs may vary between humans [10]. Generally, drug dosing and dosages are parameters that can be altered. Size of the patient is one of the main factors affecting drug dosing, although calculations of doses might be more difficult in case of obese patients [11]. This may cause problems for example in animal studies. The required dose for a small animal is significantly less than for a human.

However, it is evident that the properties of the silica-silica composite are dependent on the microparticle concentration [50]. Thus, decreasing the microparticle concentration leads to weakening mechanical properties but more importantly slower gelation as microparticles serve as nucleating agents [73][9, p. 479]. Sedimentation of microparticles will occur if the viscosity of the sol does not rise to an adequate level to prevent particle motion quickly enough [6]. As was mentioned in chapter 2.2.3, reducing microparticle concentration decreases the ability of the composite to withstand shear stresses. This may lead to phase separation in injection.

A possible solution to compensate the decreasing microparticle concentration is to lower the hydrogel R-value. Lowering the R-value reduces the amount of water in hydrogel structure and thus improves mechanical properties of the gel and could possibly stabilize the microparticles. [8, p. 126][12]

3. EXPERIMENTAL SECTION

3.1 Materials

All used silica hydrogels were synthetized during the study whereas some spray-dried microparticles were provided by DelSiTech (Finland) and others were spray-dried during the study. All microparticles were placebos: no drugs were encapsulated within the microparticles.

3.1.1 Sol-gel derived silica hydrogels

The silica sols were prepared from deionized water, 0.1 M hydrochloric acid (Titripur®, Merck Millipore) reagent grade, and 98% reagent grade tetraethoxysilane (TEOS, Sigma-Aldrich® USA). pH of the sols was adjusted with 0.1 M sodium hydroxide (NaOH, Titripur® reagent grade, Merck Millipore) and deionized water was produced with a MILLI-Q Academic (USA) water purification system. The sol-gel process is described in chapter 3.2.1.

3.1.2 Silica microparticles

Microparticles were manufactured by spray-drying of silica sol. Silica sol reagents were the same as listed in previous chapter. In addition ethanol ETAX A of 94% reagent grade (Altia, Finland) was used in dilution of the sol. Spray-drying was conducted with Büchi B-280 (Switzerland) and Büchi B-191 (Switzerland). The process parameters are presented in table 1.

Table 1. *Process parameters of spray-dried batches. RT indicates room temperature and RH room humidity*.

Date	Spray-drier	Inlet (°C)	Outlet (°C)	Aspirator (m^3/h)	Atomization (l/h)	Pump (%)	RT (°C)	RH (%)
6.6.17	Büchi B-280	120	93–96	35	670	25	-	41
22.6.17	Büchi B-191	120	66–68	35	700	25	22.5	40
26.6.17	Büchi B-191	120	93–96	35	700	25	22.5	47

Particle size distribution (PDS) was measured for the three used batches by laser diffractometry by using Sympatec Helos H2370 laser diffraction apparatus (Sympatec GmbH, Germany) (table 2).

Table 2. *Size distributions of microparticle batches used in the study. First batch (23.8.16) was provided by DelSiTech Ltd and not spray-dried during the study.*

Spay-drying date	Formulation	D_{10} (μm)	D_{50} (μm)	D_{90} (μm)
23.8.16	R5-50	2.09	7.44	18.75
6.6.17	R5-50	2.08	5.14	11.78
22.6.17	R5-50	1.80	3.90	7.59
26.6.17	R5-50	1.69	4.34	11.54

The first batch (23.8.2016) was provided by DelSiTech and the particle size distribution was not measured at the same time as of other batches.

3.1.3 Syringes and needles

The silica-silica composites were prepared at DelSiTech. Samples that were not stored for longer period than 24 hours in room temperature were prepared intp 1 ml plastic syringes supplied by Terumo or 1 ml plastic syringes supplied by BD (USA). Stored samples of hydrogels and a sample (R150, C_{mp}=0.3) stored for 2 weeks were prepared

into Plajex™ 1 ml COP plastic syringes (Terumo, Japan). Samples for shelf-life studies (R=150, C_{mp}=0.3 and 0.4 g/ml) were prepared into 1 ml plastic Gx RTF® ClearJect® (Gerresheimer, Germany).

The composite was filled into syringes. All syringes could be fitted with separately supplied hypodermic needles having a needle gauge 27 G (0.4 x 20 mm) where the first number refers to outside diameter and the latter to the length of the needle. The needles (Neolus™) were supplied by Terumo (Japan).

3.2 Methods

The goal of the study was to investigate formulations with low microparticle concentration (C_{mp}< 0.5 g/ml) and low hydrogel R-values (R < 300). Requirements for a successful formulation were that it is homogeneous, meaning evenly distributed microparticles in the hydrogel matrix with no distinct separation of the phases, injectable through a 27 G needle and maintains its viscoelastic properties for 3 months. Several formulations with R ≤ 400 and C_{mp}=0.1g/ml–0.4 g/ml were examined. Thus, the study consisted of many phases as presented in figure 11.

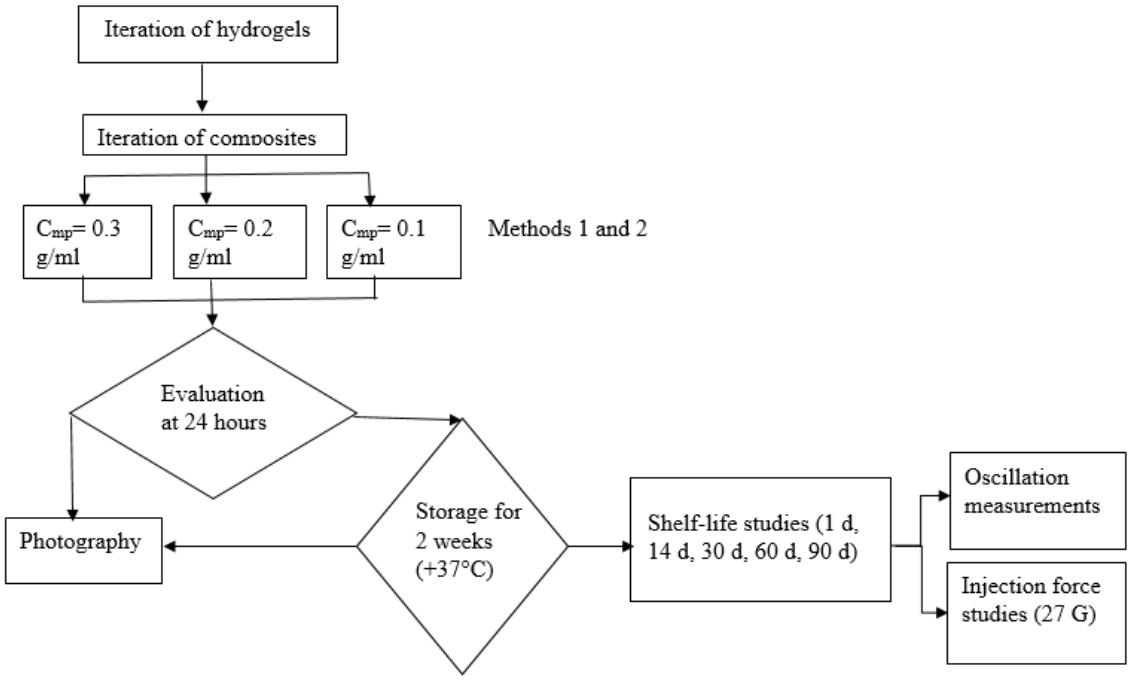


Figure 11. *A process chart of the study. The study began by selecting suitable hydrogels, proceeded to iteration of suitable formulations and the final step was to evaluate some formulations in shelf-life studies.*

The four phases of the study were the following:1) hydrogel selection, 2) study of the influence of variable microparticle concentrations on composite prepared with first method, 3) study of the influence of variable microparticle concentrations on composite prepared with second method and 4) the shelf-life study of two formulations, evaluated

with rheological and injection force studies. In hydrogel selection, hydrogels with only low R-values were studied. Firstly, a lower limit was set by preparing samples to be studied after 24-hours. Secondly, the lowest viable R-value at which the viscoelastic properties were still maintained was determined by storing three formulations for one and two weeks at +37°C.

After hydrogel R-value iteration, composite iteration was carried out to determine suitable formulations. Visual observation was conducted after stabilization of the samples (after 24 hours). If the composite was clearly heterogeneous, having two separated phases, it was photographed and no further studies were carried out. If homogenous formulations could be prepared, oscillatory measurements were conducted. One syringe was stored in +37°C for two weeks and compared to 24-hour sample to see if the composite maintained its properties. From two formulations, storage samples were manufactured for five time points (1, 14, 30, 60 and 90 days) to study the injectability and viscoelastic properties as a function of time. The stored samples were sealed in aluminum foil pouches.

Composite preparation was the main process in the study and consisted of two main procedures. Firstly, the sol was prepared by sol-gel method and secondly, the microparticles were weighed in a plastic container and sol was pipetted into the container. The mixture was stirred manually for 5 to 8 minutes to ensure homogeneity. The mixture was pipetted into plastic syringes and allowed to set in tube rotator for 24 hours. In addition to the composite preparation process, manufacturing of the silica microparticles was an additional process in the study.

3.2.1 Spray-drying of microparticles

Preparation of microparticles began as in sol-gel method. First, deionized water, 0.1 M HCL and TEOS and stirred with a magnetic stirrer (1100 rpm) for 25 minutes and then ethanol was added to the solution. The sol was produced at a mole ratio of 5:0.002:1:45, respectively. After ethanol addition, pH was adjusted with 0.1 M NaOH to approximately 6.0. The microparticles were stored at room temperature in 50 ml plastic containers.

The microparticles formulation (R5-50) was kept constant during the study. The first R-value (R5) denotes the R-value of the initial sol, before addition of ethanol. Ethanol is used to dilute the sol to higher R-value, which is indicated in the latter value in the formulation.

3.2.2 Sol-gel processing

Several formulations of silica sols were prepared in the study, ranging from R-values 50 to 400. The sols were prepared by adding deionized water, 0.1 M HCL and TEOS, at

mole ratios from 50:0.01:1 of R50 to 400:0.01:1 of R400. Approximately 20–30 ml of sol was prepared at each time.

The sol was stirred with a magnetic stirrer at 1000–1100 rpm for 25 minutes in a sealed laboratory bottle at room temperature. Stirring was done in order to speed up hydrolysis and to ensure that it is complete. The object was to retain vigorous stirring, without splashing. Mixing was successful if the solution had only one phase in the end of stirring. After stirring, pH of the sol was approximately 2 in which silica is metastable and the condensation proceeds very slowly. pH of the sol was raised to 6.2 (±0.1) with 0,1 M NaOH. The sol could be directly pipetted into a syringe to study only the hydrogel.

3.2.3 Composite preparation

Composite preparation began after the sol was set to pH 6.2±0.1. At this point, condensation proceeds rapidly [70] and aging of the sol was considered to begin. The aim was to use fresh sols which have not been allowed to age before further processing: for this reason, mixing of the sol with microparticles began immediately. Some experiments were conducted with aged sols which will be discussed in chapter 4.2.1. The composites were prepared with two alternative methods.

In the first method, microparticles were weighed in a 50-ml plastic container. Microparticle mass concentrations (C_{mp}) of the prepared composites were 0.1 g/ml; 0.2 g/ml and 0.3 g/ml. In addition, some stored samples were manufactured with C_{mp}= 0.4 g/ml. The sol was pipetted into the container with microparticles and the composite was mixed for approximately 5 to 8 minutes with 5 ml pipette head, to produce a homogenous mixture. Composite amount was at the beginning approximately 1 ml but the amount was increased to 3 to 4 ml; to ensure facile stirring. Next, 0.8-1 ml of composite was filled into syringes and held in a tube rotator for 24 hours at 7 rpm to prevent microparticle sedimentation before stabilization. Stabilization indicates the time after sedimentation of microparticles no longer occurs. 24 hours is calculated from the point a syringe is placed into the tube rotator as is all time-points in the study. The 24-hour time point was set based on Noppari's studies [86] where it has been shown that a composite with formulation R400 C_{mp}=1 g/ml stabilizes in 8 hours, indicating that 24 hours should be adequate. Filling and placing a syringe into the tube rotator took approximately 3-5 minutes.

In the second method, the sol was prepared in the same way as in the first method and the pH was raised to 6.2±0.1. Microparticles were weighed in a 50 ml CELLSTAR® tube (Greiner Bio-One, Austria) and their total mass was weighed. Next, microparticles were washed with 20 ml of sol and the system was mixed with a Vortex mixer (Fischer Scientific, USA). The solution was centrifuged in 4000 rpm for 10 minutes and excess sol was removed with a pipette. Microparticles absorbed some sol which is why all sol could not be removed. Total mass of the container and its contents were weighed and

the volume of the absorbed sol was calculated. Next, more sol was added to obtain the desired microparticle concentration within the composite. The composite was then mixed manually with a 5 ml pipette head for 5 minutes and filled into 1 ml syringes. The syringes were placed in a tube rotator with 7 rpm and allowed to stabilize for 24 hours. The studied formulations and preparation method are given in table 3.

Table 3. *Prepared composite formulations in the study and their preparation method. Number 1 or 2 indicate the manufacturing method (method 1 (1) or method 2 (2). A hyphen indicates that a sample was not prepared.*

R-value	C_{mp}=0.1 g/ml	C_{mp}=0.2 g/ml	C_{mp}=0.3 g/ml	C_{mp}=0.4 g/ml
R125	1	1	1	-
R150	1	1	1	1
R200	-	1	1	-
R250	-	-	1	-
R300	-	2	2	-
R400	-	2	2	-

Some formulations were chosen for shelf-life studies and the samples were studied with oscillatory and injection force measurements in five time points. Consequently, 20 samples of one formulation were prepared, so that four samples could be studied in each time point. Due to manual manufacturing of the syringes, the sol may have significantly aged as last samples were prepared. Thus, two identical batches of sol were prepared so only 10 samples were prepared from each batch of sol, to minimize the aging of the sol.

3.2.4 Rheological measurements

Rheological measurements were conducted with ThermoHaake RS 300 (Germany) rotational rheometer with a parallel-plate geometry HPP20 TC measuring geometry (D=20 mm). Oscillatory measurements were conducted in controlled deformation and with two measuring settings. The storage sample for 14 days and the 24-hour reference sample were measured with 0.4 gap, deformation of 0.002 and frequency range of 0.01-10 Hz. The samples of the shelf-life study were measured with 1 mm gap, deformation of 0.001 and frequency range of 0.1-10 Hz.

3.2.5 Injection force measurements

Injection force studies were conducted with Lloyd-Ametek LS100 Plus (USA) with a 250 N load cell. Needle gauge was kept constant at 27 G. The measured machine extension was converted into amount of injected composite.

4. RESULTS AND DISCUSSION

4.1 Hydrogel selection

The study of silica hydrogels was conducted to determine a hydrogel with lowest possible R-value that could be utilized in composite preparation. Hydrogels with low R-values have two characteristics that may prevent their use in composite preparation. Firstly, decreasing R-value increases the solid substance in the hydrogel and possibly weakens the injectability. In addition, microparticles further stiffen the composite and thus, if the hydrogel itself has low injectability, composite preparation would not be necessary. Secondly, decreasing R-value could result in continuous condensation reaction and structural changes. [30, p. 368]

4.1.1 24-hour evaluation

Preparation of the hydrogels began with R-values R50, R100 and R200. The 24-hours samples were evaluated by visual observation. 24 hours indicates time when the sample was left to gel and the moment of examination. Surface properties and structure of the gel were evaluated by visual observation. Table 5 shows the terms that were used to characterize the samples.

Table 5. *Characterization of hydrogels. Surface properties, structure and injected product were evaluated based on presented characterizations.*

Surface properties	**Structure**
Glossy	Solid gel; not letting liquid out
Matte	Weak gel; some liquid separated
Grainy	Liquid-like
Smooth	

R100 could be described as glossy, grainy and solid gel. R200 was also glossy, but represented a smooth weak gel: the structure was runny. R50 was considerably different

than R100 and R200: the hydrogel was matt and grainy. In addition, gelation occurred fast, within first 10 minutes with 10 ml of R50 which could be problematic in composite preparation. Therefore, R50 was ruled out of the study and replaced with R80 in further studies.

4.1.2 Stored hydrogel samples

Samples of R80, R100 and R150 were prepared for storage in +37°C and the results are tabularized in table 6.

Table 6. *Characterization of hydrogel samples stored for two weeks. The injections were performed through a 27 G-needle manually. Injectability is described as poor, ok or good, based on the required force to push the plunger.*

	1 week		2 weeks	
R-value	**Injectability**	**Visual observation**	**Injectability**	**Visual observation**
R80	Poor, few droplets	Matt, grainy, solid gel. Separation of liquid.	Poor, few droplets	Matt, grainy, solid gel,
R100	Good	Glossy, grainy, solid gel.	Poor	Glossy, grainy, solid gel. Separation of liquid.
R150	Good	Glossy, smooth, weak gel	Good	Glossy, smooth, weak gel

Both R80 and R100 went through structural changes that altered their appearance and injectability. At two weeks' time point, both R100 and R80 were notably "dry" and came out of the syringe as separate pieces instead of a uniform gel. R150 did not change its properties dramatically in visual observation. Thus, R100 and R80 were ruled out of the studies. However, the lowest R-value in the composite was set to R125 because R150 had no dramatic structural changes while the changes of R100 were not as obvious as of R80.

4.2 Iteration of homogeneous composite formulations

Preparation of composites with different R-values and microparticle concentrations was conducted to find out homogeneous formulations that maintain their viscoelastic properties in short time storage test. These samples could be studied further in shelf-life studies for longer storage period.

4.2.1 Short term stability samples

The 24-hour samples were the first evaluation method of composites. The main goal was to study whether visually homogenous samples could be prepared from certain formulations. The results are reported as sample photograph. The first samples were prepared with preparation method 1, utilizing fresh sol directly pipetted to microparticles Figure 12 presents the samples of R125.

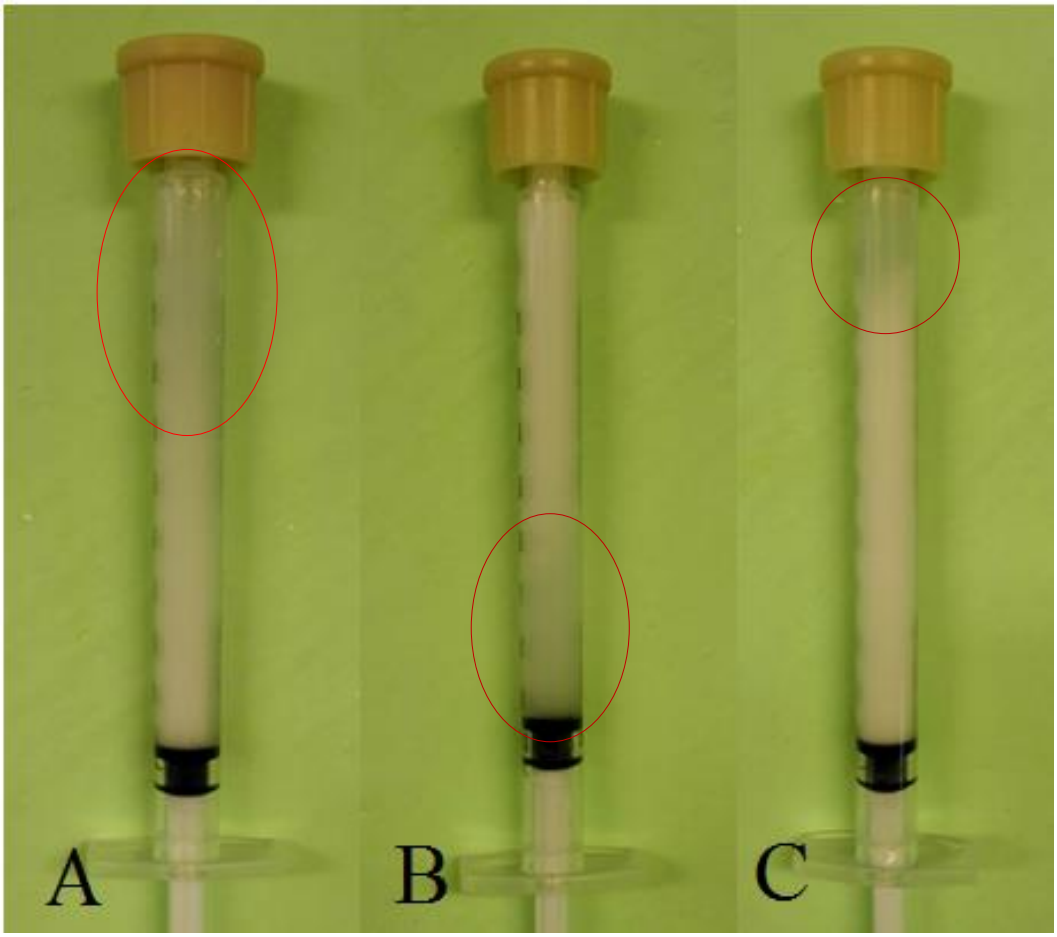


Figure 12. *Samples of R125. From the left, the samples present different microparticle concentrations: 0.1 g/ml (A), 0.2 g/ml (B) and 0.3 g/ml (C). Circled areas indicate separation in a sample.*

The samples showed clearly inhomogeneous structure. The circled areas in Figure 12 indicate areas with only hydrogel and no microparticles. That could be seen as an obvious change of color: a homogeneous syringe would be evenly white. In all samples, a clear sedimentation of microparticles occurred. The hydrogel area was smallest in sample with C_{mp}=0.3 g/ml and slightly smaller in sample with C_{mp}=0.2 g/ml than in sample with C_{mp}=0.1 g/ml which is logical since the lowest concentration has the lowest amount of microparticles and also pack in a smaller volume. The hydrogel areas were either on top or bottom of the syringe which indicates the effect of rotation pushing the microparticles on either end. The syringes where randomly placed in the tube rotator

either pointing up or down and the microparticles were packed also randomly on either end of the syringe, regardless of the syringe position.

Increasing R-value showed more homogeneous samples at the highest applied concentration. Samples of R150 are presented in figure 13.

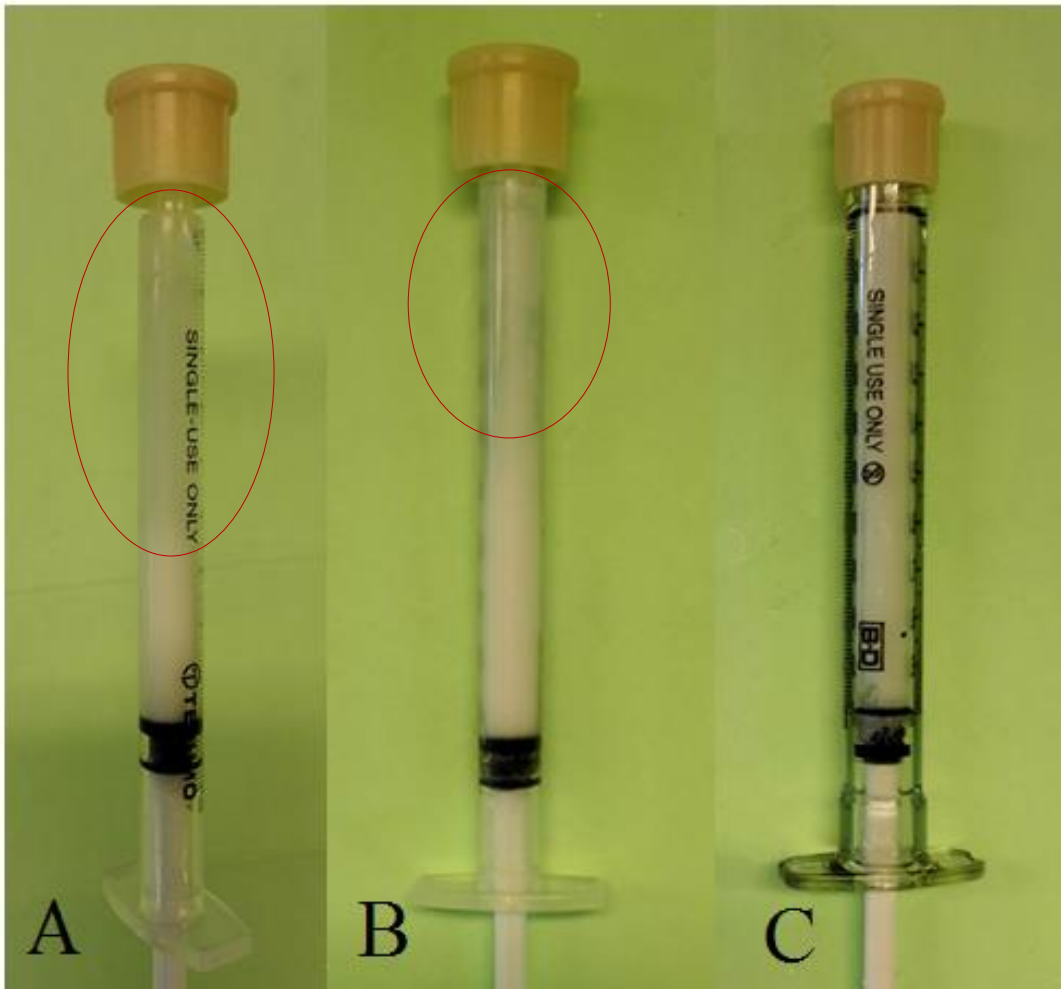


Figure 13. *Samples of R150. From the left, the samples present different microparticle concentrations: 0.1 g/ml (A), 0.2 g/ml (B) and 0.3 g/ml (C). Circles areas indicate hydrogel separation.*

With these samples, a significant difference between different microparticle concentrations could be observed. A sample with C_{mp}=0.1 g/ml was mostly consisting of hydrogel and a small volume of packed microparticles near the piston. Again, sample with C_{mp}= 0.2 g/ml had considerably smaller volume of separated hydrogel but the sample was still heterogeneous. On the other hand, a sample with C_{mp}= 0.3 g/ml is visually homogenous. The color is uniform throughout the syringe area and, despite of a small separated area near the piston, the sample showed the most homogeneity of all prepared samples. A small separation was more likely to be caused by an air bubble or unsuccessful filling than the material property.

Despite of a successful sample of R150, increasing the R-value even more did not improve the homogeneity of the samples. Figure 14 shows the samples prepared of R200 and R250.

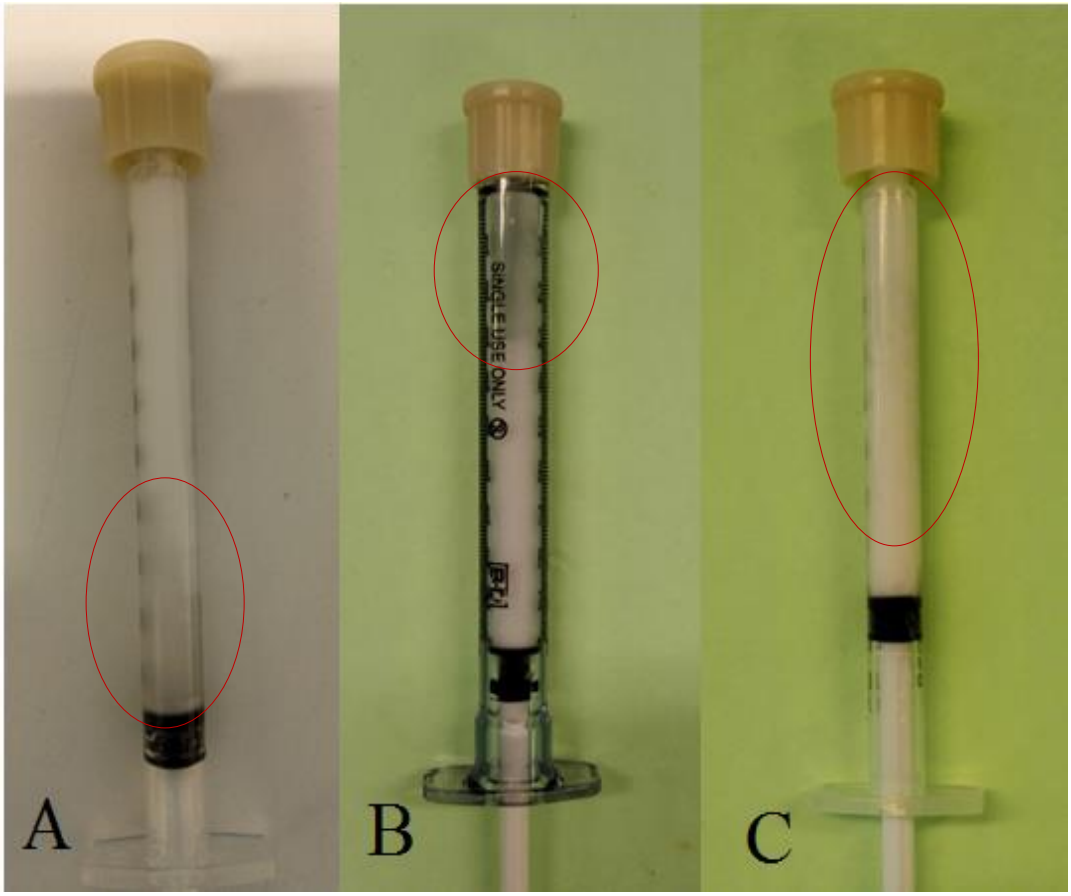


Figure 14. *Samples of R200 and R250. From the left, the samples present different formulations: R200 and C_{mp}=0.2 g/ml (A), R200 and C_{mp}=0.3 g/ml (B) and R250 and C_{mp}=0.3 g/ml (C). Circled areas indicate hydrogel separation.*

All samples were again heterogeneous. Samples with C_{mp}= 0.1 g/ml were not prepared because the concentration was not successful at lower R-values and an increasing R-value would result in weaker gel with increased gelation time [8, p. 126][12]. Thus, R200 was prepared with C_{mp}=0.3 g/ml and 0.2 g/ml and R250 only with C_{mp}=0.3 g/ml. Samples of R200 were clearly heterogeneous with clear hydrogel area but the sample of R250 showed a different structure. The sample was heterogeneous but had small hydrogel areas all over the syringe. The pattern is similar to a marble surface: hydrogel areas and microparticle clusters formed a random pattern. In addition, as all studied samples were injected from the syringe without a needle, clear microparticle clusters could be observed in most of the samples.

After the preparation with method 1, some samples were prepared with the alternative method 2. In this method, microparticles were prewetted with sol in order to stabilize

them and prevent them from sedimentation. The method was suggested by DelSiTech since it was used in microencapsulation. R-values of 300 and 400 were used in composite preparation since R400 has been shown to maintain viscoelastic properties for 30 days' storage time. [86] Figure 15 shows samples prepared with R300.

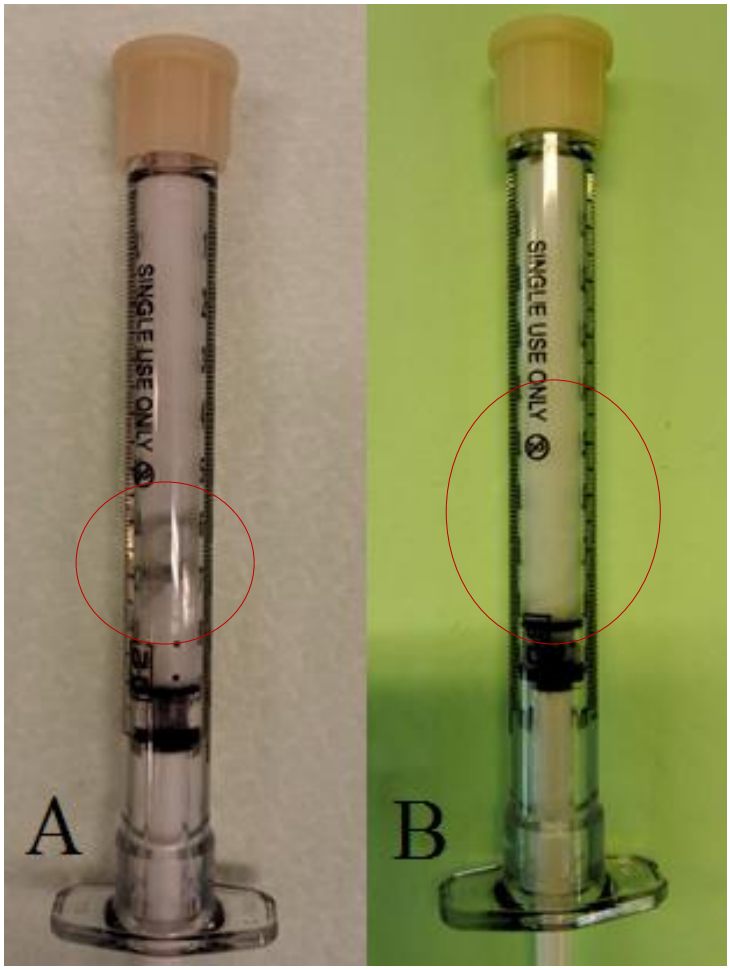


Figure 15. *Samples of R300. From the left, C_{mp}=0.2 g/ml (A) and C_{mp}=0.3 g/ml (B). Circled areas indicate separated hydrogel.*

A sample with C_{mp}=0.2 g/ml was heterogeneous and separation had concentrated near the piston. The position indicated that an air bubble was trapped in the syringe. However, hydrogel areas were also visible on other areas of the sample. Separation in sample with C_{mp}=0.3 g/ml was not as obvious but hydrogel areas were also present in the syringe.

Samples with R400 were also prepared with the method 2. Respectively, the samples showed clear heterogenicity (figure 16).

Figure 16. *Samples of R400. From the left, C_{mp}=0.2 g/ml (A) and C_{mp}=0.3 g/ml (B). Circled areas indicate hydrogel separation.*

Both samples had separated hydrogel areas, as the samples of R300.

The reason why sedimentation of microparticles occurred can be derived from the microstructure and rheology. The reason why the composite is manually mixed for a certain time is that viscosity drops as shearing takes place in the material, allowing particles to be homogenously mixed to the sol [46, p. 21]. As mixing is ended, the original structure is restored [6][7] which means also the same viscosity value as before mixing. Sedimentation occurred in almost all samples, indicating also that with lowered R-value condensation does not proceed quickly enough to raise viscosity to an adequate level [6]. Gravitation affects the microparticles [53] and lack of viscosity causes the sedimentation. This confirms that microparticles act as seeds of nucleation [9, p. 479]. With higher microparticle concentrations, gelation proceeds more quickly and prevents particle sedimentation.

As already studied in DelSiTech, aging of the sol – meaning the waiting time before sol is mixed to the microparticles as pH is risen to 6.2 – was beneficial in composite preparation. Effect of aging was studied with some formulations, such as R150 C_{mp}=0.1 g/ml and R200 C_{mp}=0.3 g/ml (figure 17).

Figure 17. *Samples prepared with aged sol. Figure A: R150 Cmp=0.1 g/ml, aged in room temperature for 10 minutes before adding microparticles. Figure B: R200 Cmp=0.3 g/ml, aged for 60 minutes in room temperature before adding microparticles.*

Visually homogeneous samples could be conducted even with C_{mp}=0,1 g/ml. However, the optimum aging time is dependent on the sol volume and R-value and is thus difficult to predict.

As a conclusion, preparing homogeneous samples from fresh sols was proven to be challenging. The samples were very sensitive and for example increasing rotation speed resulted in heavy sedimentation of microparticles. However, it must be noted that experiments were conducted with only one microparticle formulation and the effects of microparticle size, geometry or encapsulated drugs were not evaluated.

4.2.2 Samples stored for two weeks

In this study, it was essential to evaluate the shelf-life of the homogeneous formulations. The prefilled syringes are designed to be "ready-to-use" products which can be injected at desired time after storage. Thus, it is important that the composite maintains its viscoelastic properties. One sample of R150 C_{mp}=0.3 g/ml was prepared in storage in +37°C and compared to a 24 h-sample. If remarkable changes in the structure would

already occur in two weeks, it would be useless to prepare several samples for various time points for 3 months' storage. The G' and G'' values obtained from oscillation measurements could be compared between a 24-hour sample and a two week-sample (figure 18).

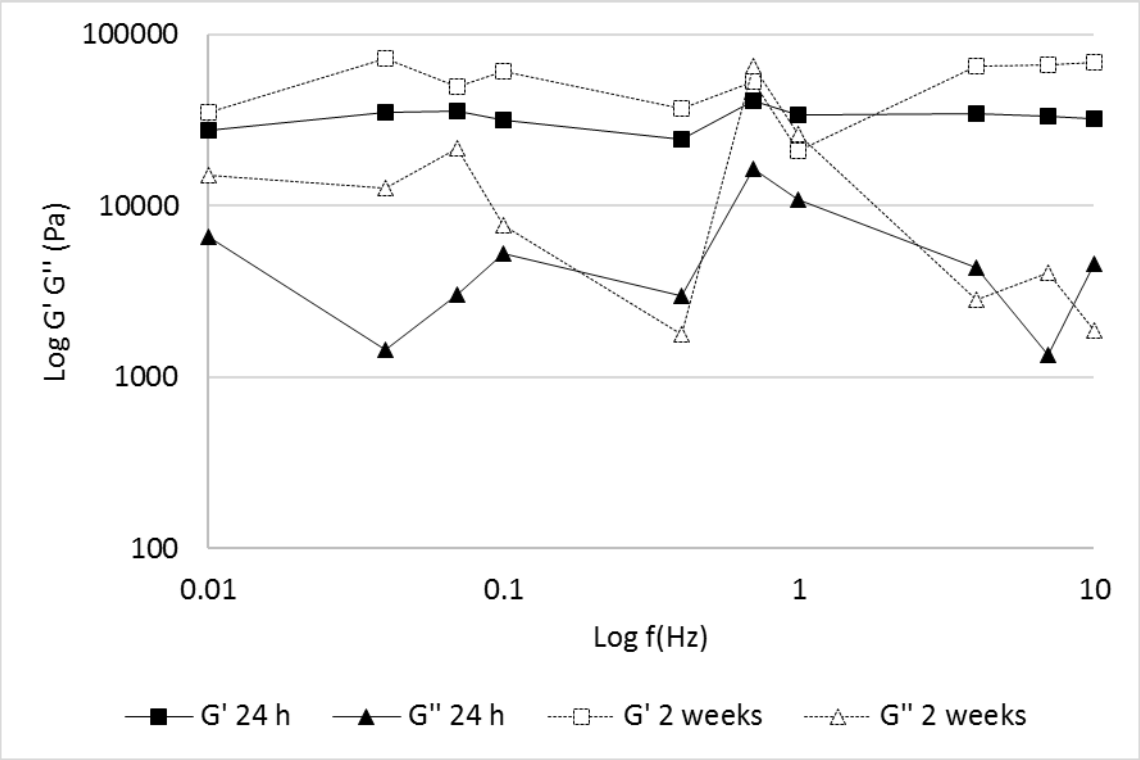


Figure 18. *The effect of two weeks' storage on R150 C_{mp}=0.3 g/ml. The G'- and G''-values are presented for a sample stored for 24 hours in room temperature and for a sample stored for two weeks at +37°C. The plotted values are averages of three replicate measurements.*

G' values did not significantly increase indicating that no remarkable condensation has occurred within two weeks' time [8, p. 389]. In ideal case of a homogeneous hydrogel, the G' and G'' diagrams would be almost horizontal. Both samples thus indicated some inhomogeneity. However, it must also be noted that there were only three replicate measurements and due to the sensitivity of the material and difficult insertion between the plates in measurements, absolute repeatability is difficult to obtain.

4.3 Shelf-life of R150

Since R150 C_{mp}=0.3 g/ml possessed no significant structural changes in two weeks, samples for storage were manufactured to evaluate the evolution of composite injectability and structure within 3 months. The samples were visually evaluated. Since the formulation R150 C_{mp}=0,3 g/ml showed inhomogeneity in samples stored for 24 hours and 2 weeks, additional samples for storage were prepared on R150 C_{mp}=0.4 g/ml. The decision was also supported by the fact that there were no other successful formulations

for shelf life studies and although the most preferable microparticle concentrations were 0.1 g/ml, 0.2 g/ml and 0.3 g/ml, the final limit was set as C_{mp} < 0.5 g/ml. Figure 19 shows the prepared samples of R150 C_{mp}=0.3 g/ml and 0.4 g/ml.

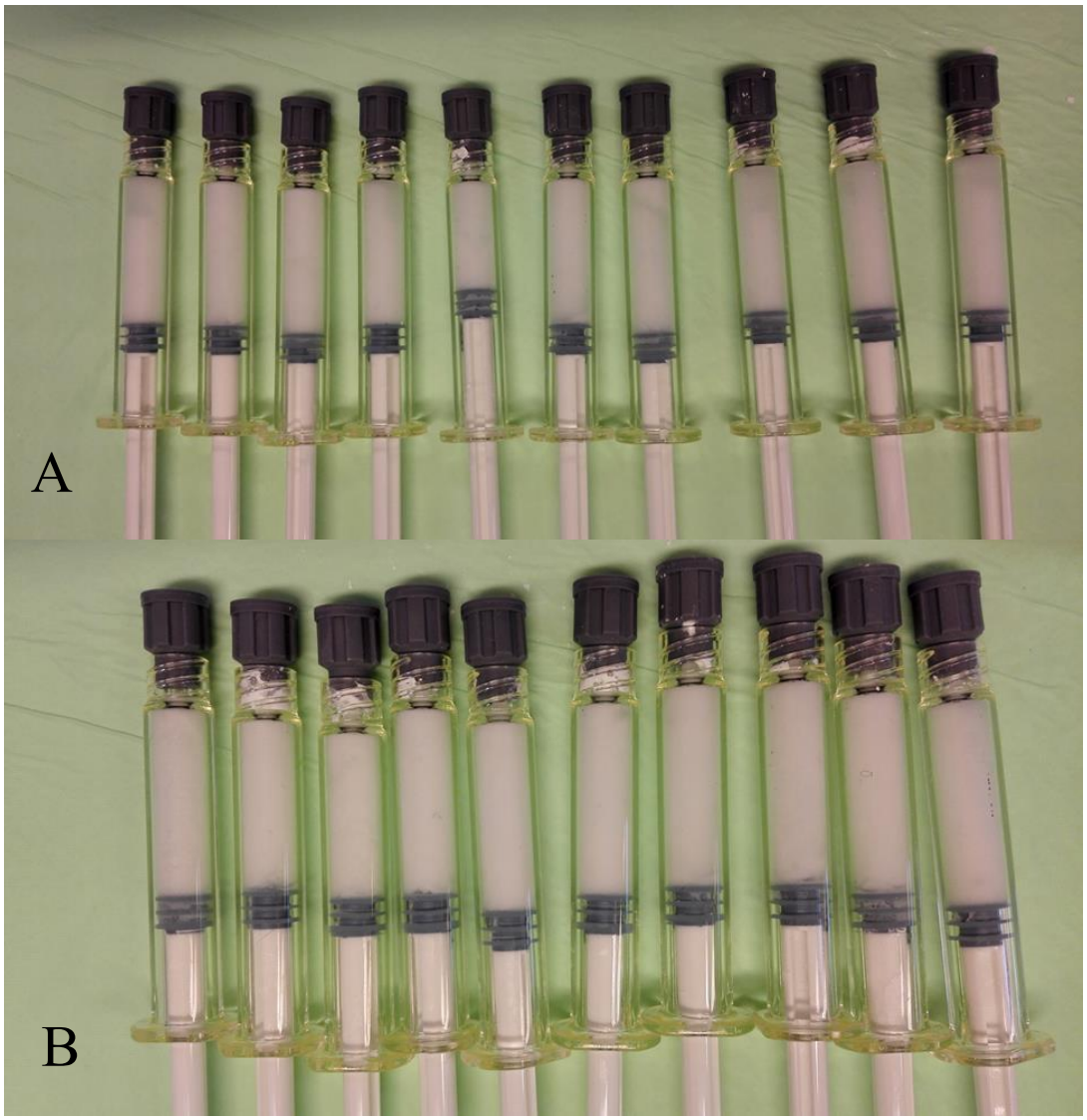
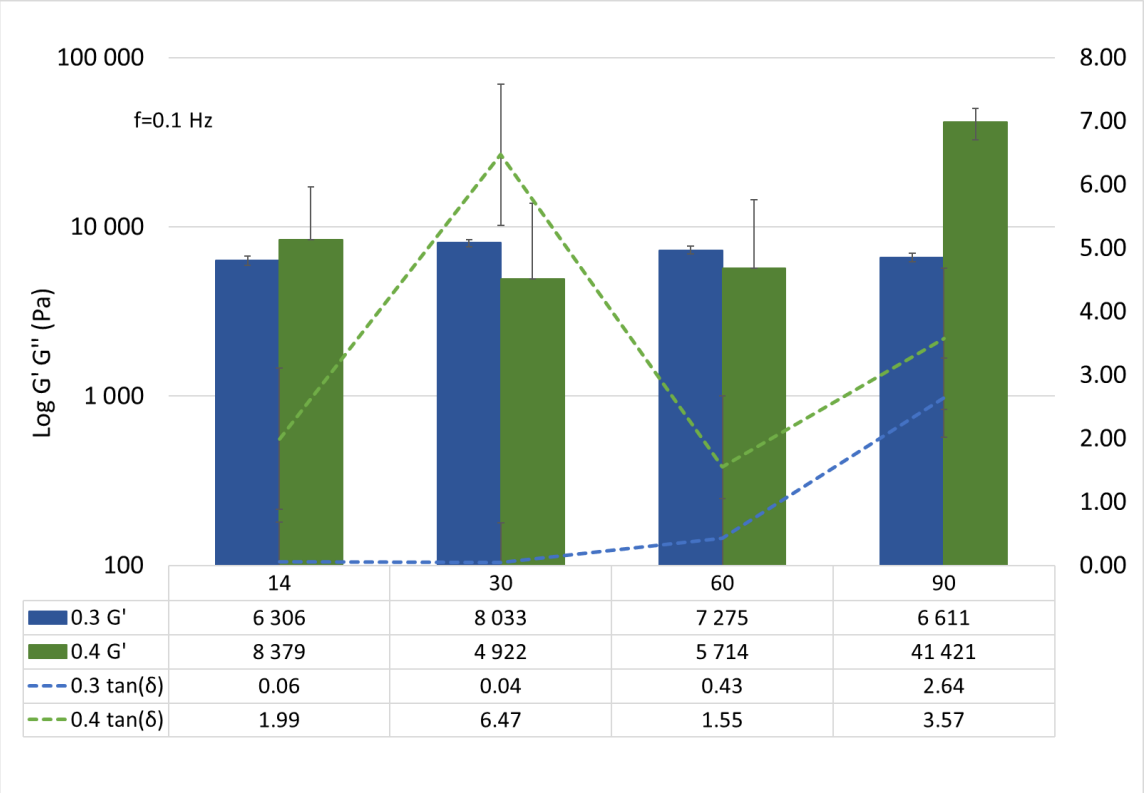


Figure 19. *Samples of R150 for shelf-life studies. Figure A presents samples stabilized for 24 hours in room temperature with C_{mp}=0.3 g/ml and figure B samples with 0.4 g/ml.*

The prepared samples with C_{mp}=0.4 g/ml were visually more homogenous than C_{mp}=0.3 g/ml. Separated hydrogel areas could be observed in most of the syringes, while some were relatively homogeneous. This also indicates that obtaining identical samples of the formulation is challenging although the filling method, sol age and preparation method were kept constant.

4.3.1 Viscoelasticity of the stored samples

In order to investigate the structure of the composite and its homogeneity, rheological measurements were conducted in small angle oscillatory shear. Figure 20 shows the results in 0.1 Hz. G'- and G''-values are plotted in each time point. The results are shown in two different frequencies, 0.1 Hz and 10 Hz. These frequencies are the starting and ending frequency in frequency sweep measurements.



	14	30	60	90
0.3 G'	6 306	8 033	7 275	6 611
0.4 G'	8 379	4 922	5 714	41 421
0.3 tan(δ)	0.06	0.04	0.43	2.64
0.4 tan(δ)	1.99	6.47	1.55	3.57

Figure 20. *G'- and G''-values in 14, 30, 60 and 90 in days' time points measured in 0.1 Hz. The plotted values are averages of six replicate measurements.*

G'-values of samples with C_{mp}=0.3 g/ml did not possess significant differences in 90 days. However, tan(δ) has risen, indicating higher G''-values. This again suggests structural changes in the composite. As G''-values exceed the G'-values (tan(δ)>1) viscous properties are dominating in the composite, suggesting liquid-like behavior. [62, p. 359, 363]

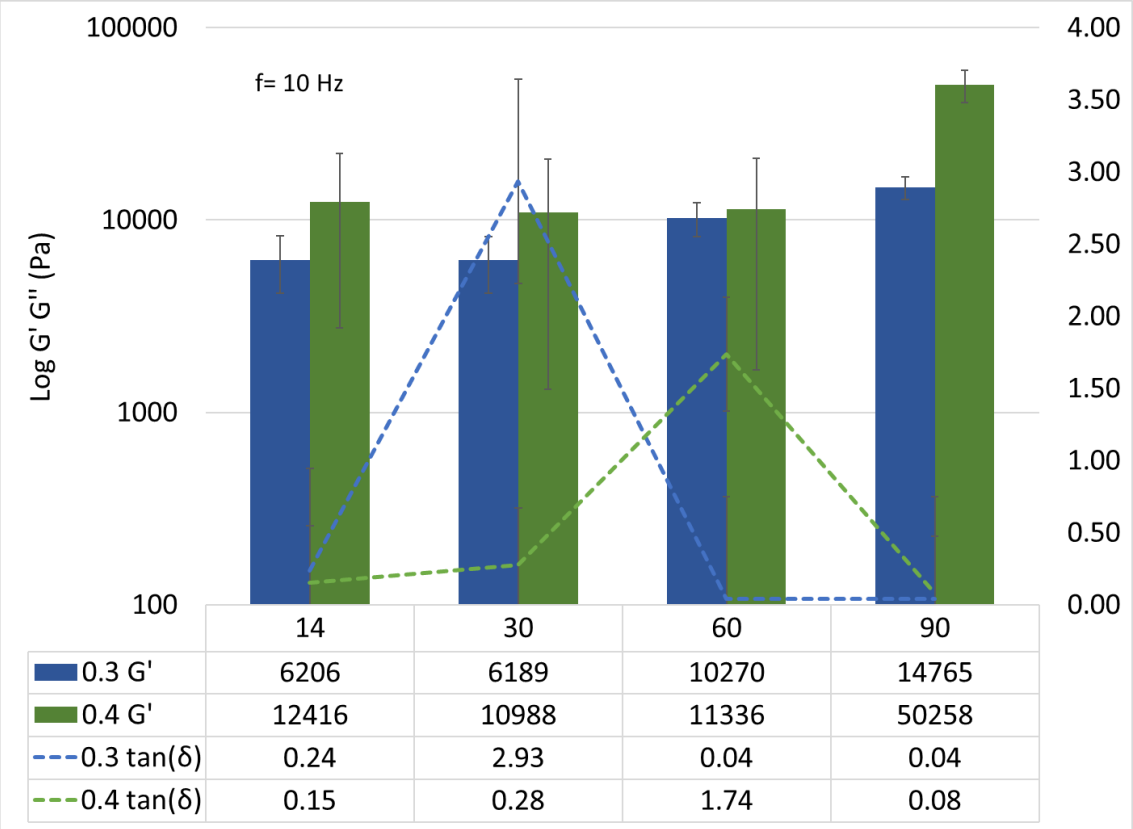
Samples with C_{mp}=0.3 g/ml possessed higher G'-values in 30 and 60 days' time points than samples with C_{mp}=0.4 g/ml although in particulate composites the particles should stiffen the material. [50] Samples with C_{mp}=0.3 g/ml were observed to let out liquid as they were compressed between the plates, but the same phenomenon was not observed with samples with C_{mp}=0.4 g/ml. Thus, G'-values are higher since some amount of liquid is leached out of the hydrogel structure.

In 90 days, significant structural changes were observed in some of the samples. In case of C_{mp}=0.4 g/ml, the G'-values rise significantly in three months' time point: G'-values had risen since some of the samples had turned into a monolith (figure 21).

Figure 21. *Some of three months' samples possessed significant structural changes. Figure A is a sample with C_{mp}=0.4 g/ml and figure B is a sample with C_{mp}=0.3 g/ml. The samples have turned into shrunken, solid mass and some liquid has concentrated on the barrel walls.*

This phenomenon was observed in several syringes, 3/8 samples in total. Figure 21 showed the most dramatic example of syneresis, the leakage of water from the pores and solidification of the composite with C_{mp}= 0.4 g/ml in 90 days' time point. [62, p.359] This sample affects the G'-values significantly since in other syringes the phenomenon is not as dramatic. In one sample with C_{mp}=0.3 g/ml and in one sample with C_{mp}=0.4 g/ml syneresis was shown as slight shrinkage of the composite and concentrated water in the barrel walls.

Figure 22 shows the results for 10 Hz which also indicated aging phenomena.



	14	30	60	90
0.3 G'	6206	6189	10270	14765
0.4 G'	12416	10988	11336	50258
0.3 tan(δ)	0.24	2.93	0.04	0.04
0.4 tan(δ)	0.15	0.28	1.74	0.08

Figure 22. *G'- and G''-values in 14, 30, 60 and 90 in days' time points measured in 10 Hz. The plotted values are averages of six replicate measurements.*

In 10 Hz, the G'-values showed more obvious increase in both formulations. The samples with C_{mp}=0.3 g/ml did not show an increase of tan(δ)-values in 90 days as in 0.1 Hz. This suggests that in rest, the sample has liquid "pockets" and the viscous behavior is more prominent. As shearing takes place and the sample deforms, the pockets move and G''-values decrease.

Aging of the composite can be accelerated by temperature but also by rising pH-levels. Especially coarsening is strongly pH-dependent. [30, p. 364] The pH-values were measured from depots before filling into syringes at the manufacturing stage and in 90 days' time point (table 7).

Table 7. *pH values of stored samples. pH was measured from non-aged microparticle/ sol mixture (stored for 0 days) and samples stored at +37°C for 90 days.*

R-value	C_{mp} (g/ml)	Batch	Storage time (d)	pH
R150	0.3	1	0	6.0
R150	0.3	2	0	6.0
R150	0.4	1	0	5.9
R150	0.4	2	0	6.0
R150	0.3	1	90	5.8
R150	0.3	2	90	5.8
R150	0.4	1	90	5.7
R150	0.4	2	90	5.7

It is evident that the pH-values do not explain the structural changes: the values stayed within the same range.

Overall, samples with C_{mp}=0.3 g/ml were visually more inhomogeneous than samples with C_{mp}=0.4 g/ml and let out liquid in injection. However, samples with C_{mp}=0,4 g/ml had more variation between replicate measurements. As a result, with R150 all samples did not maintain their viscoelastic properties for 90 days. Aging, especially syneresis was observed in both formulations. However, not all samples turned into monoliths and with C_{mp}=0.3 g/ml most samples did not alter significantly. Overall, 3/8 samples in three months' time point had shrunk in to a solid mass and let out water from their structure. The result indicates some differences between the samples since not all samples possessed as dramatic structural changes despite they were identically prepared. The differences may arise from manufacturing of the syringes, for example successfulness of the filling. However, this also indicates that the samples are prone to aging. Additionally, longer storage times may result in more structural changes in the composite, as low R-value is utilized [62, p. 389].

4.3.2 Injectability of the stored samples

Injectability of the composites was studied through a 27 G-needle. A fine needle was selected since it is applicable even in intraocular injections [83]. The shear-thinning characteristic allows the composite to be injected through a fine needle [6][7] The results are given separately for both formulations and graphs plotted as the amount inject-

ed out as a function of the applied force is presented in supplements (figures 23–27). The results for samples with C_{mp}=0.3 g/ml are presented (table 8).

Table 8. *Injectability of R150 C_{mp}=0.3 g/ml samples. Injectability is presented as the amount of composite that was injected out of 1000 µl. Observations indicate whether all of the composite was injected out (successful) or not (blocked).*

Storage time (d)	Amount injected out (µl)	Observations
1	241	Blocked
	267	Blocked
14	477	Blocked
	954	Successful
30	858	Blocked
	679	Blocked
60	889	Successful
	945	Successful
90	363	Blocked
	437	Blocked

In most cases, injectability of the samples was poor: 5/10 syringes were blocked before half of the composite was injected out. In addition, 80–100 N that can be considered as a rough estimation of the maximum finger pressing force for an adult was exceeded [57]. Only 3/10 injections were successful meaning the whole dose is injected out of the syringe. No trend could be observed between different batches. However, the injected amount of composite was notably smaller in 1 day and 90 days' time point than in other time points. The poor injectability in 90 days was supported by rheological and visual observations: many samples had significantly solidified. The results in the 24 hours' time point are more likely to be caused by the measuring technique. The measurements were manually stopped if the needle was blocked. In some occasions, microparticles may form bigger clusters at the syringe entry and block the syringe for few seconds [52]. This can be seen in injection force measurements as sudden force peaks. The integrated structure may be broken down quickly and most probably the first measurements were stopped too early in case of such an obstruction.

Both samples in 60 days' time point were successfully injected. The same phenomenon can be observed with C_{mp}=0.4 g/ml samples (table 9).

Table 9. *Injectability of R150 Cmp=0.4 g/ml samples. Injectability is presented as the amount of composite that was injected out of 1000 µl. Observations indicate whether all of the composite was injected out (successful) or not (blocked).*

Storage time (d)	Amount injected out (µl)	Observations
1	700	Blocked
	269	Blocked
14	688	Blocked
	589	Blocked
30	632	Blocked
	451	Blocked
60	911	Successful
	927	Successful
90	902	Successful
	427*	Successful

*The syringe was not filled to 1000 µl

The explanation for the improved injectability can be derived from sample selection since no obvious trend of improved injectability of both formulations could be observed or supported from rheological studies. These differences may arise from aging. The samples were manufactured in two batches to minimize the aging time of the sol. However, it took approximately 3-5 minutes to fill a syringe and place in a tube rotator. This means that when five samples were manufactured, the last syringe was filled from a sol that had been aging for already 12-20 minutes. As was seen in figure 17A, already 10 minutes of aging time improved the homogeneity of the syringe with formulation R150 C_{mp}=0.1 g/ml.

Overall, injectability of samples with C_{mp}=0.4 g/ml was also poor, although only 2/10 samples were blocked before half of the composite was injected. This supports the visual observation that samples with C_{mp}=0.3 g/ml are more heterogeneous than samples with C_{mp}=0.4 g/ml although more variation is seen in oscillatory measurements. A total of 4/10 samples were successfully injected, including one syringe that was not filled to

1000 µl. The results indicate that inhomogeneous structure is present in both formulations and causes difficulties in injections.

The results indicate that the material is not flowing consistently but phase separation occurs by filtration in the barrel. As the plunger is pushed in the syringe, the hydrogel matrix starts to flow more easily. If the material is heterogeneous, microparticles may form bigger clusters or be already packed close as hydrogel matrix flows. The integrated microparticles may cause a blockage at the barrel [52]. It is evident that the formulations do not withstand the pressure as being injected through a fine needle. Based on the results of this study, lower R-values than R150 are not recommended for composite preparation. However, higher R-values would result in weaker mechanical properties of the matrix [12] and thus the ability to withstand the injection may decrease.

5. CONCLUSIONS

The aim of this study was to examine if a formulation with lowered microparticle concentration could be successfully manufactured (C_{mp} < 0.5 g/ml). A successful formulation was defined by three characteristics: the composite must be homogeneous, injectable through 27 G-needle and maintain its viscoelastic properties for three months. Low microparticle concentrations are required in animal testing since composites are formulated for humans but they are first studied in animals. Since the lowered microparticle concentration weakens the mechanical properties of the composite and increases heterogeneity, R-value was also lowered, ranging from R50 to R400 in order to improve the mechanical properties of the matrix.

At the beginning, an iteration of suitable R-values was conducted by visual observation of fresh hydrogel samples and those which had been stored in +37°C to enhance the aging process for one and two weeks. After the shelf-life study of R80, R100 and R200, both R80 and R100 had structural changes: in both formulations, the liquid was leached out of the hydrogel structure. The result was supported by self-injection studies through a 27 G-needle in which R100 and R80 showed poor injectability in 2 weeks' time. As a conclusion, an R-value between R100 and R150 is acting as the lowest limit for a hydrogel that still maintains its structure or in other words remarkable condensation does not occur in two weeks' time in +37 °C.

Iteration of suitable formulations for shelf-life studies, ranging from R125–R400 with C_{mp}=0.1–0.4 g/ml, showed that a homogeneous sample with low microparticle concentrations was difficult to obtain. In most cases, sedimentation of the microparticles occurred in 24 hours although a tube rotator was used during stabilization. Thus, lowered R-value does not sufficiently compensate the decreased microparticle concentration and viscosity does not rise to an adequate level to stabilize the microparticles. However, only one microparticle formulation was used in studies and the effect on size or shape was not investigated. The only formulation from which visually homogeneous samples could be prepared was R150 C_{mp}=0.3 g/ml. However, some visual inhomogeneity could be observed as several samples were manufactured for storage.

Shelf-life studies of R150 C_{mp}=0.3;0.4 g/ml were conducted with five measuring points for three months. This was important since it was already clear with hydrogels that aging may induce significant structural changes that may destroy viscoelastic characteristics of the composite. In oscillatory measurements, this phenomenon was proven: total of 3/8 samples had shrunk and released liquid from the pores after three months. Aging processes were evidently occurring in both formulations and rising G'-values could be

observed with $C_{mp=}$0.4 g/ml. With many samples of C_{mp}=0.3 g/ml, liquid was separated in the syringe and the G''-values had increased. However, some samples had less dramatic structural changes and thus variation between identical measurements was increased during time. Injection force studies showed poor injectability of the formulations: Only 2/10 injections with C_{mp}=0.3 g/ml and 4/10 injections with C_{mp}=0.4 g/ml were successful. In addition, the results showed that there was notable variation between the samples. This in turn indicates that some samples had been prepared from aged sol which improves the homogeneity. As a result, R150 is possibly near the lowest limit of usable R-values in composite preparation and no lower R-value is recommended. Some of the samples did not maintain the viscoelastic properties and longer storage time could enhance the structural changes.

Aging of the sol evidently improves the homogeneity of the sols. From fresh sols, a homogeneous formulation that keeps the viscoelastic properties was not successfully manufactured. Thus, for C_{mp}<0.5 g/ml another approach is required. Since the aging times are difficult to predict for different volumes, a successful formulation could be manufactured by adding the microparticles to an already formed hydrogel with capability to avoid microparticle sedimentation. The hydrogel exhibits shear-thinning characteristics which may be used to mix the microparticles within the gel (exceeded the gel-point). A system such as dual-barrel syringe could potentially offer a solution, by adding only sol to other barrel and sol with microparticles to another. Extrusion would provide mixing of both barrel content to obtain a composite with light concentration. A dual-barrel syringe can be used to dispense or inject fluids [90]. However, hydrogels with R-values lower than 150 are not recommended in composite preparation since aging processes were clearly observed with R150. Another approach to obtain homogeneous formulations is to use additives in the hydrogel network to stabilize the microparticles and to obtain homogenous structure. Glycerol has been studied with silica aerogels to increase the gelling time and to cause an increase in G'-values [91]. The effect of glycerol has been studied to be beneficial in PVA hydrogel, by improving physical properties [92]. Another polyol additive could also be used. Linseed oil based polyol has been studied as a potential crosslinking agent that leads to the formation of interpenetrating networks and also introducing a hydrophobic covering over a polymeric matrix [93].

The main objective in future research regarding lowered microparticle concentrations is to focus on improving of the properties of the silica hydrogel matrix. Rapid increase in viscosity in the matrix is essential in stabilizing the microparticles. As a conclusion, either additives in the hydrogel matrix or adding microparticles to a gel could be investigated in the future in order to obtain composites with low microparticle concentrations.

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SUPPLEMENTS

Figures of injection force measurements of stored formulations R150 C_{mp}=0.4;0.3 g/ml are presented below. The results are presented for five time points (1 d, 14 d, 30 d, 60 d, 90 d). The amount of composite injected out (µl) is plotted as a function of injection force (N). The maximum finger press force of an adult is approximately 100 N. [57]

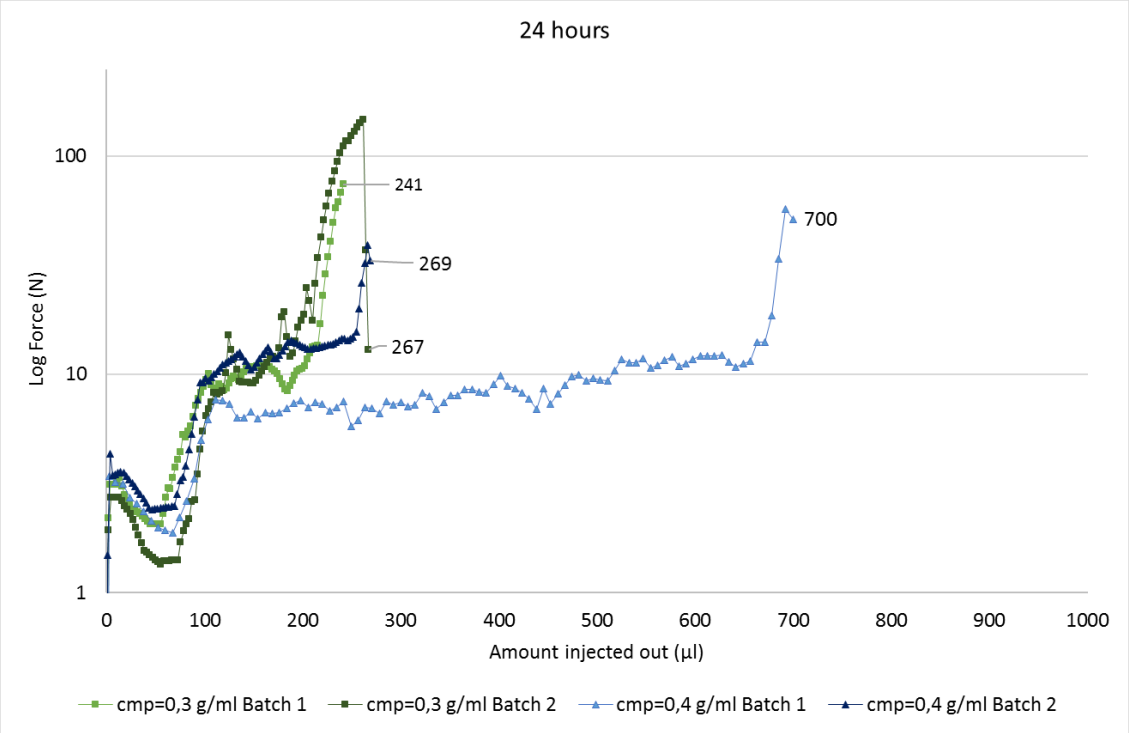

Figure 23. *Injection force measurements of R150 C_{mp}=0.3 g/ml and 0.4 g/ml stored for 24 hours in room temperature. Two syringes of both formulations were measured and the filling of the syringes was 1000 µl. All syringes were blocked.*

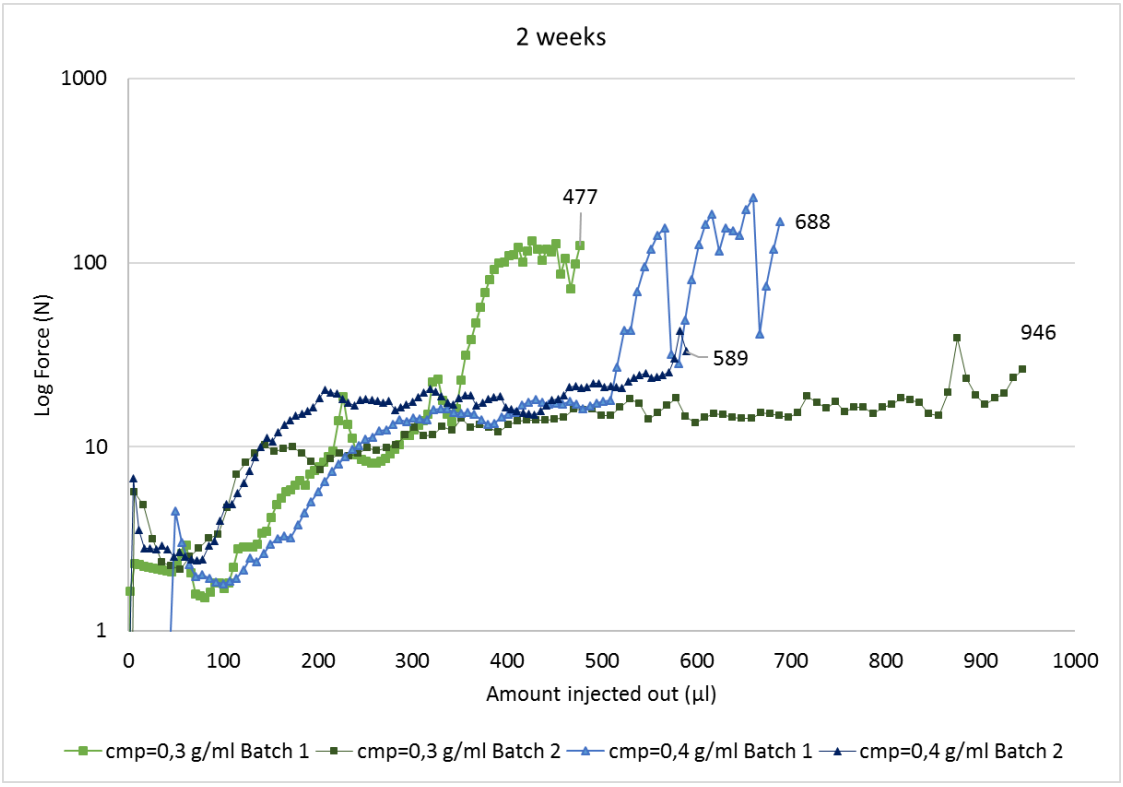

Figure 24. *Injection force measurements of R150 C_{mp}=0.3 g/ml and 0.4 g/ml stored for two weeks in +37°C. Two syringes of both formulations were measured and the filling of the syringes was 1000 µl. One injection was successful.*

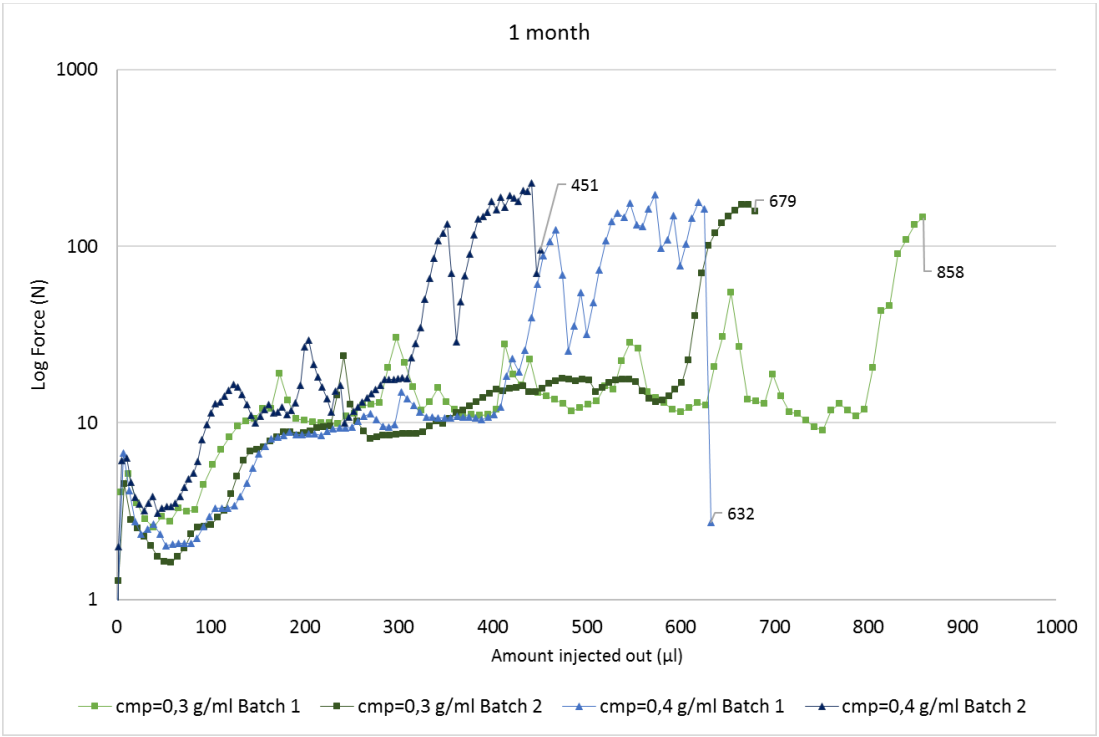

Figure 25. *Injection force measurements of R150 C_{mp}=0.3 g/ml and 0.4 g/ml stored for one month in 37°C. Two syringes of both formulations were measured and the filling of the syringes was 1000 µl. All syringes were blocked.*

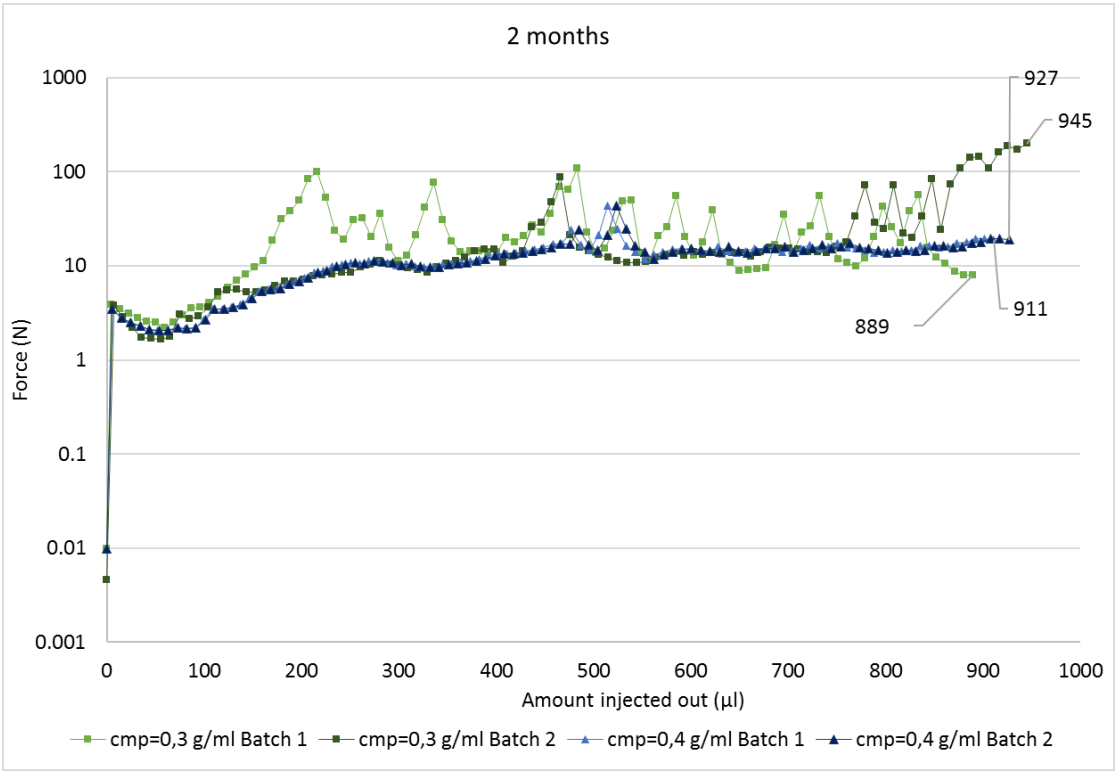

Figure 26. *Injection force measurements of R150 C_{mp}=0.3 g/ml and 0.4 g/ml stored for two months in +37°C. Two syringes of both formulations were measured and the filling of the syringes was 1000 µl. All injections were successful.*

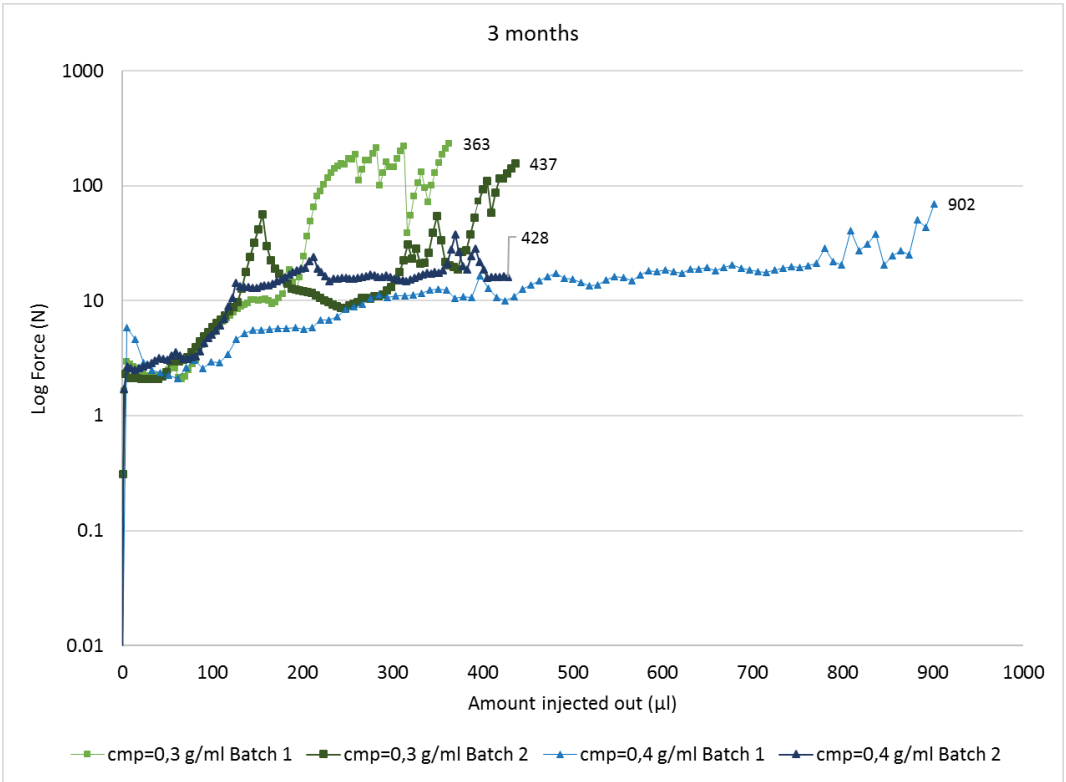

Figure 27. *Injection force measurements of R150 C_{mp}=0.3 g/ml and 0.4 g/ml stored for three months in +37°C. Two syringes of both formulations were measured and the filling of the syringes was 1000 µl. Two injections were successful.*